Dimensional Analysis of the Model of the Wnt Pathway

Starting with the complex, detailed description of a system, dimensional analysis searches for the simplest mathematical form to represent the real system (Deen, 1998). In complex systems, dimensional analysis can usually result in a dramatic simplification of the problem. A side effect of performing the dimensional analysis is that during the process, the parameters in the system automatically rearrange into groups of parameters, called the dimensionless groups. The number of dimensionless groups indicates the degrees of the freedom in the system, and is often smaller than the actual number of physical parameters. A well-known example of a dimensionless group is the Reynolds number.

(To imagine the concept of parameter grouping, consider for example, a Michaelis-Menten reaction. What dictates the rate of product formation is effectively the value of $K_M$, not the individual rate constant per se.)
Dimensional analysis is performed by scaling the variables in the system such that the scaled variables are:

1. Dimensionless
2. In the order of 1 (written as “\(\sim 1\)”).

Let \(X\) be a variable in the system. We will search for a scale \(X_0\), which has the same unit as \(X\), and define the dimensionless variable \(x\) as

\[
x \equiv \frac{X}{X_0} \sim 1
\]

In practice, a variable is appropriately scaled if its maximum value is not too small (<0.1) or too large (>10).

The scale \(X_0\) can be found using the order-of-magnitude analysis (Deen, 1998), which we illustrate next. As will become apparent shortly, this process involves some guessing and intuitive reasoning about how the system operates. One control for this process is self-consistency. There can be several appropriate scales for a given variable. If we derive a “wrong” scale, the scaled variable will simply be either very large (>10) or very small (<0.1), \(i.e.,\) not in the order of 1. But in general, we can scale the problem any way we like; some scales may yield more insights than others, depending on the questions we ask.

Our starting point was a system of 7 ordinary differential equations (ODEs) and 8 algebraic equations, derived in (Lee et al., 2003). The dimensional analysis could have been performed on the original problem of 15 ODEs; With respect to steady-state solutions, we will obtain the same results. To facilitate the discussion, the 7 ODEs are reproduced here:

\[
\frac{dX_2}{dt} = k_1 W (D_{vl} - X_2) - k_2 X_2 \quad (1)
\]
\[
\frac{dX_9}{dt} = \frac{k_9 X_3 X_{11}}{K_8} - k_{10} X_9 \quad (2)
\]
\[
\frac{dX_{10}}{dt} = k_{10} X_9 - k_{11} X_{10} \quad (3)
\]

\[
\frac{dX_4}{dt} = -(k_3 X_2 + k_4 + k_6) X_4 + k_5 X_3 + k_6 X_5 \frac{K_{17} X_{12} \cdot APC}{K_7(K_{17} + X_{11})} \quad (4)
\]

\[
\frac{dX_{12}}{dt} \left(1 + \frac{APC \cdot K_{17}}{K_7(K_{17} + X_{11})}\right) = \frac{dX_{11}}{dt} \frac{APC \cdot K_{17} X_{12}}{K_7(K_{17} + X_{11})^2} = k_3 X_2 X_4 - k_6 \frac{GSK \cdot APC \cdot K_{17} X_{12}}{K_7(K_{17} + X_{11})} + k_{-6} X_4 + v_{14} - k_{15} X_{12} \quad (5)
\]

\[
\left(1 + \frac{X_{11}}{K_8}\right) \frac{dX_3}{dt} + \frac{X_3}{K_8} \frac{dX_{11}}{dt} = k_4 X_4 - k_3 X_3 - \frac{k_9 X_3 X_{11}}{K_8} + k_{10} X_9 \quad (6)
\]

\[
\frac{dX_{11}}{dt} \left(1 + \frac{X_3}{K_8} \frac{TCF \cdot K_{16}}{(K_{16} + X_{11})^2} + \frac{APC \cdot K_{17}}{(K_{17} + X_{11})^2}\right) + \frac{X_{11}}{K_8} \frac{dX_3}{dt} = v_{12} - \left(\frac{k_9 X_3}{K_8} + k_{13}\right) X_{11} \quad (7)
\]

where the parameters are defined in Figure 2A, and the variables are:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>X_2</td>
<td>Active Dvl</td>
</tr>
<tr>
<td>X_3</td>
<td>APC*/Axin*/GSK3</td>
</tr>
<tr>
<td>X_4</td>
<td>APC/Axin/GSK3</td>
</tr>
<tr>
<td>X_9</td>
<td>β-catenin*/APC*/Axin*/GSK3</td>
</tr>
<tr>
<td>X_{10}</td>
<td>β-catenin*</td>
</tr>
<tr>
<td>X_{11}</td>
<td>β-catenin</td>
</tr>
<tr>
<td>X_{12}</td>
<td>Axin</td>
</tr>
</tbody>
</table>

(* = phosphorylated)
Finding Concentration Scales: Order-of-Magnitude Analysis

As an illustration, we now derive the concentration scale for β-catenin, $X_{110}$. The most intuitive guess is that the concentration of β-catenin scales with its steady-state value. Let us set eqn. 7 to zero, and solve for $X_{11}$,

$$X_{11} = \frac{v_{12}}{k_9 X_3 + k_{13}}$$

(8)

We make a guess that β-catenin scales with its steady-state value,

$$X_{110} \sim \frac{v_{12}}{k_9 X_{30} + k_{13}}$$

(9)

where $X_{30}$ is the scale for $X_3$.

Since we have a sense that the rate of non-Axin-dependent degradation $\ll$ rate of Axin-dependent degradation,

$$k_{13} \ll \frac{k_9 X_{30}}{K_8}$$

(10)

we can safely simplify eqn. 9 to,

$$X_{110} \sim \frac{v_{12} \cdot K_8}{k_9 X_{30}}$$

(11)

To further support our intuition, from the Xenopus parameters,

$$k_{13} \sim 10^{-4} \quad \frac{k_9 X_{30}}{K_8} \sim 10^{-3}$$

$$10^{-3} + 10^{-4} \sim 10^{-3}.$$

To fully derive the concentration scale of β-catenin, we need to find $X_{30}$. We find $X_{30}$ using a similar procedure:

1. Estimate the order of magnitude of APC*/axin*/GSK3 concentration ($X_3$) at steady state (set equations 2,4-6 to zero)
(2) Perform an order-of-magnitude substitution (as in eqn. 9)
(3) Drop the small terms (as in eqns. 11)

If we “mistakenly” drop terms, we will simply get an inappropriate scaling. In which case, we go back to the order-of-magnitude analysis and try keeping or dropping another term, until we find the appropriate concentration scales that make the scaled variable ~ 1.

After some algebraic steps, we have the following expression for $X_{30}$,

$$X_{30} = \frac{k_4}{k_5} \cdot \frac{k_6 \cdot GSK3 \cdot (APC/K7) \cdot (v_{14}/k_{15})}{k_{-6}}$$

(12)

Substituting eqn. 12 into eqn. 11, we have the concentration scale for β-catenin.

Performing, the order-of-magnitude analysis for the other variables, we obtain the following concentration scales:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_2$</td>
<td>Active Dvl</td>
<td>$X_{20} = Dvl$</td>
</tr>
<tr>
<td>$X_3$</td>
<td>APC*/Axin*/GSK3</td>
<td>$X_{30} = \frac{k_4}{k_5} \cdot \frac{k_6 \cdot GSK3 \cdot (APC/K7) \cdot (v_{14}/k_{15})}{k_{-6}}$</td>
</tr>
<tr>
<td>$X_4$</td>
<td>APC/Axin/GSK3</td>
<td>$X_{40} = \frac{k_5}{k_4} \cdot X_{30}$</td>
</tr>
<tr>
<td>$X_9$</td>
<td>β-cat*/APC*/Axin*/GSK3</td>
<td>$X_{90} = \frac{v_{12}}{k_{10}}$</td>
</tr>
<tr>
<td>$X_{10}$</td>
<td>β-catenin*</td>
<td>$X_{100} = \frac{v_{12}}{k_{11}}$</td>
</tr>
<tr>
<td>$X_{11}$</td>
<td>β-catenin</td>
<td>$X_{110} = \frac{v_{12} \cdot K_8}{k_9 \cdot X_{30}}$</td>
</tr>
<tr>
<td>$X_{12}$</td>
<td>Axin</td>
<td>$X_{120} = \frac{v_{14}}{k_{15}}$</td>
</tr>
</tbody>
</table>

(* denotes a phosphorylated state)
Scaling the Model of the Wnt Pathway

After finding the concentration scales, the next step is to scale the variables. As an illustration, we now scale the ODE describing the dynamics of $\beta$-catenin (eqn. 7). To simplify the algebra, let us consider the steady-state problem:

$$0 = v_{12} - \left( \frac{k_g X_3}{K_8} + k_{13} \right) X_{11}$$

(13)

Let us define $x_{11}$ as the dimensionless $\beta$-catenin concentration,

$$x_{11} \equiv \frac{X_{11}}{X_{110}}$$

where $X_{11}$ is $\beta$-catenin concentration, $X_{110}$ is the concentration scale of $\beta$-catenin. Similarly, we define $x_3$ as the dimensionless APC*/Axin*/GSK3 concentration.

Substituting $x_{11}, X_{110}$ for $X_{11}$ (sometime called non-dimensionalizing the equation),

$$0 = v_{12} - \left( \frac{k_g X_{30}}{K_8} x_3 + k_{13} \right) \frac{v_{12} K_8}{k_g X_{30}} x_{11}$$

(14)

Let us rearrange eqn. 14 into its simplest form, by dividing both sides with $k_{13} : K_{17}$

$$0 = \frac{v_{12}}{k_{13} : K_{17}} - \left( \frac{k_g X_{30}}{K_8 : k_{13}} x_3 + 1 \right) \frac{v_{12} K_8}{k_g X_{30} : K_{17}} x_{11}$$

(15)

Let us now define the following dimensionless groups,

$$\alpha = \frac{k_g X_{30}}{K_8 : k_{13}}$$

(16)

$$\gamma = \frac{v_{12}}{k_{13} : K_{17}}$$

(17)

Substituting these into eqn. 15, we obtained a dimensionless equation describing $\beta$-catenin (eqn. 20 below).
Performing a similar scaling procedure to eqns. 1-6 at steady state, we obtain the set of dimensionless ODEs describing the Wnt pathway at steady-state.

**The dimensionless ODEs describing the Wnt pathway at steady state:**

\[ 0 = \gamma - (\alpha x_3 + 1) \frac{\gamma}{\alpha} x_{11} \]  \hspace{1cm} (18)

\[ 0 = \delta_1 (1 - x_2) + x_2 \]  \hspace{1cm} (19)

\[ 0 = x_9 - x_3 \cdot x_{11} \]  \hspace{1cm} (20)

\[ 0 = x_9 - x_{10} \]  \hspace{1cm} (21)

\[ 0 = (\delta_2 x_2 + 1) x_4 - \frac{x_{12}}{1 + \frac{\gamma}{\alpha} x_{11}} \]  \hspace{1cm} (22)

\[ 0 = x_3 - x_4 \]  \hspace{1cm} (23)

\[ 0 = x_{12} - 1 \]  \hspace{1cm} (24)

The four dimensionless groups describing the system are:

<table>
<thead>
<tr>
<th>Dimensionless groups</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>( \frac{k_9 X_{30}}{K_8 \cdot k_{13}} )</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>( \frac{v_{12}}{k_{13} \cdot K_{17}} )</td>
</tr>
<tr>
<td>( \delta_1 )</td>
<td>( \frac{k_1 \cdot W}{k_2} )</td>
</tr>
<tr>
<td>( \delta_2 )</td>
<td>( \frac{k_3 \cdot Dvl}{k_{-6}} )</td>
</tr>
</tbody>
</table>

With respect to \( \beta \)-catenin, the groups \( \delta_1 \) and \( \delta_2 \) merge further:

\[ \delta = \frac{k_3 \cdot Dvl}{k_{-6}} \cdot \frac{k_1 \cdot W}{1 + k_1 \cdot W/k_2} \]
The original problem (eqns. 1-7) has been systematically rescaled such that scaled variables are now in the order of 1. In the process, the numerous parameters automatically rearrange into 4 groups of parameters.

Each parameter in the model corresponds to a physical, measurable parameter (e.g., protein concentration, rate constants, equilibrium constant, etc.), describing interactions known to take place in the Wnt pathway. Dimensional analysis reveals that many of these parameters affect the system in the same way: there are only 4 independent ways to perturb the model of the Wnt pathway at steady state. We now derive an analytical solution for the model.

Steady-State Solution of the Model of the Wnt Pathway

We now solve the scaled model (eqns. 18-24) for:

1. The steady-state level of $\beta$-catenin
2. The Wnt-induced fold-change in $\beta$-catenin

From eqn. 18, the steady-state ODE describing $\beta$-catenin is

$$0 = \gamma - (\alpha x_3 + 1) \frac{\gamma}{\alpha} x_{11} \quad (18)$$

To solve for $\beta$-catenin ($x_{11}$), we derive $x_3$ from eqns. 19-24,

$$x_3 = \frac{1}{\left(1 + \frac{\gamma}{\alpha} x_{11}\right)(1 + \delta)} \quad (25)$$

Substituting eqn. 25 into eqn. 18,

$$0 = \gamma - \left[\frac{\alpha}{\left(1 + \frac{\gamma}{\alpha} x_{11}\right)(1 + \delta)} + 1\right] \frac{\gamma}{\alpha} x_{11} \quad (26)$$

After a series of algebraic steps, eqn. 26 simplifies into a quadratic form,
\[ 0 = \frac{\gamma}{\alpha} x_{11}^2 + \left[ 1 - \gamma + \frac{\alpha}{(1 + \delta)} \right] x_{11} - \alpha \]  

(27)

There are two possible solutions for a quadratic equation. Since \( \beta \)-catenin concentration has to be \( \geq 0 \), we can eliminate the negative solution. The positive solution for eqn. 27 is the steady-state, dimensionless concentration of \( \beta \)-catenin:

\[
x_{11} = \frac{1 - \gamma + \frac{\alpha}{1 + \beta}}{2\gamma/\alpha} \left( \frac{4\gamma}{1 + \left(1 - \gamma + \frac{\alpha}{1 + \delta} \right)^2} - 1 \right)
\]

(28)

This can be converted back compute the concentration of \( \beta \)-catenin (in nM) by multiplying both sides by \( X_{110} \).

The dimensional, steady-state concentration of \( \beta \)-catenin (nM):

\[
X_{11} = K_{117} \cdot \frac{1 - \gamma + \frac{\alpha}{1 + \delta(Wnt)}}{2} \left( \frac{4\gamma}{1 + \left(1 - \gamma + \frac{\alpha}{1 + \delta(Wnt)} \right)^2} - 1 \right)
\]

(29)

where \( \delta \) is proportional to the Wnt concentration:

- If \( Wnt \neq 0 \), \( \delta = \delta(Wnt) \)
- If \( Wnt = 0 \), \( \delta = 0 \)

To compute the Wnt-induced fold-change in \( \beta \)-catenin, we take the ratio of Wnt-induced level of \( \beta \)-catenin and basal level of \( \beta \)-catenin.
The Wnt-induced fold-change in $\beta$-catenin:

\[
F = \frac{X_{11}(\delta)}{X_{11}(0)} = \frac{1 - \gamma + \frac{\alpha}{1 + \delta(Wnt)}}{1 - \gamma + \alpha} \sqrt{\frac{1}{1 + \frac{4\gamma}{(1 - \gamma + \alpha)^2} - 1}}
\]  

Equations 29-30 were used to generate Figures 3A-B.

Incorporating the Dual role of APC (Takacs et al., 2008)

It was recently shown that APC has a negative role (promoting degradation of $\beta$-catenin) and a positive role in Wnt signaling. APC most likely promotes degradation of Axin. This interaction was considered in the published study by (Lee et al., 2003) (Figure 5 and eqn. 5). In the present analysis, this addition simply leads to extra parameters in $\alpha$.

Current model:

\[
\alpha = \frac{k_{g} \cdot k_{T} \cdot k_{6} \cdot GSK3\beta \cdot APC \cdot v_{14}}{K_{5} \cdot k_{13} \cdot K_{6} \cdot k_{6}}
\]

With APC promoting degradation of Axin:

\[
\alpha = \frac{k_{g} \cdot k_{T} \cdot k_{6} \cdot GSK3\beta \cdot APC \cdot v_{14}}{K_{5} \cdot k_{13} \cdot k_{6} \cdot K_{7} \cdot K_{M} + APC}
\]

The blue circle indicates where the Axin degradation term now contains APC. The remaining analysis remains the same, just a re-definition of $\alpha$. 
Understanding the Mechanism of Robustness Using Separation of Timescale

To gain more insights, we derive an approximate description of $\beta$-catenin accumulation by using separation of time scales in the system. Because of the slow DC-independent degradation, $\beta$-catenin is the sluggish component in the system. (DC is short for destruction complex, APC/Axin/GSK3) The other six independent variables reach steady state relatively rapidly, and are subsequently slaved by the slow dynamics of $\beta$-catenin, until the level of $\beta$-catenin reaches a steady state a few hours later.

The ODE describing $\beta$-catenin dynamics is given in eqn. 7,

$$\frac{dX_{11}}{dt} \left(1 + \frac{X_3}{K_8} + \frac{TCF \cdot K_{16}}{(K_{16} + X_{11})^2} + \frac{APC \cdot K_{17}}{(K_{17} + X_{11})^2}\right) + \frac{X_{11}}{K_8} \frac{dX_3}{dt} = v_{12} - \left(\frac{k_9 X_3}{K_8} + k_{13}\right) X_{11}$$ (7)

Setting $\frac{dX_3}{dt} = 0$ and rearranging,

$$\frac{dX_{11}}{dt} = \frac{v_{12} - \left(\frac{k_9 X_3}{K_8} + k_{13}\right) X_{11}}{1 + \frac{X_3}{K_8} + \frac{TCF \cdot K_{16}}{(K_{16} + X_{11})^2} + \frac{APC \cdot K_{17}}{(K_{17} + X_{11})^2}}$$ (33)

Setting eqns. 1-6 to zero and solving for $X_3$,

$$X_3 = \frac{X_{30}}{\left(1 + \frac{X_{11}}{K_{17}}\right) \left(1 + k_i W/k_2\right)}$$ (34)

where $X_{30}$ is the concentration scale for $X_3$, as derived in the previous section.
Substituting eqn. 34 into eqn. 33,

\[
\frac{dX_{11}}{dt} \approx v_{12} \left( \frac{k_9}{K_8} \left( 1 + \frac{X_{11}}{K_{17}} \right) \left( \frac{X_{30}}{1 + \frac{k_2Dvl}{k_{-6}} \cdot \frac{k_1W/k_2}{1 + k_1W/k_2}} \right) + k_{13} \right) X_{11}
\]

\[
(35)
\]

The denominator in eqn. 35 is only significantly different from 1 within the initial transient period. Since we are examining the behavior at and near steady state here, it can therefore be set to 1.

We are left with the following dimensional, approximate equation describing \(\beta\)-catenin dynamics,

\[
\frac{dX_{11}}{dt} \approx v_{12} \left( \frac{k_9}{K_8} \left( 1 + \frac{X_{11}}{K_{17}} \right) \left( 1 + \frac{k_2Dvl}{k_{-6}} \cdot \frac{k_1W/k_2}{1 + k_1W/k_2} \right) + k_{13} \right) X_{11}
\]

\[
(36)
\]

Let us now scale eqn. 36, using the following dimensionless variables,

\[
x_{11} = \frac{X_{11}}{X_{110}}; \quad \tau = \frac{t}{1/k_{13}}
\]

where \(X_{110}\) is the concentration scale for \(\beta\)-catenin derived in the previous section, and \(k_{13}\) is the rate constant of DC-independent degradation of \(\beta\)-catenin (this is the slow step in b-catenin dynamics).
Substituting the scaled variables, and the dimensionless groups defined in the previous section, we obtain the dimensionless, approximate ODE of $\beta$-catenin dynamics:

$$\frac{dx_{i1}}{d\tau} \approx \alpha - \frac{\alpha}{1 + \delta} \cdot \frac{x_{i1}}{1 + \frac{\gamma}{\alpha} x_{i1}} - x_{i1}$$  \hspace{1cm} (37)

Let us deconstruct eqn. 37 to understand what dictates the dynamics of $\beta$-catenin:

$$\frac{dx_{i1}}{d\tau} \approx \alpha - \frac{\alpha}{1 + \delta} \cdot \frac{x_{i1}}{1 + \frac{\gamma}{\alpha} x_{i1}} - x_{i1}$$  \hspace{1cm} (38)

**Positive feedback term**  
(~$\beta$-cat-APC binding)

The dynamics of $\beta$-catenin is affected by an effective production term and two degradation terms: the DC-dependent degradation (regulated by ligand, represented by the dimensionless group $\delta$) and the DC-independent degradation. The binding between $\beta$-catenin and APC (reaction 17) appears as a positive feedback term in eqn. 38: it inhibits the DC-dependent degradation term. In the *Xenopus* system, binding between $\beta$-catenin and APC is weak ($K_d \sim 1000\text{nM}$). But under appropriate condition (when $\gamma/\alpha$ is large enough), this interaction can act as a positive feedback: $\beta$-catenin sequesters APC, amplifying the effects of Wnt in inactivating the destruction complex. (Notice that it is not enough to simply make the binding stronger, equivalent to increasing $\gamma$, but $\alpha$ also has to be smaller than $\gamma$ for the positive feedback to appear).
The steady-state solution of eqn. 38 is simply the steady-state solution of the full problem, given in eqn. 28. And the Wnt-induced fold-change in β-catenin is derived before in eqn. 30:

\[
F = \frac{X_{11}(\delta)}{X_{11}(0)} = \frac{1 - \gamma + \frac{\alpha}{1 + \delta}}{1 - \gamma + \alpha} \sqrt{\frac{4\gamma}{1 + \frac{\alpha}{1 + \delta}}} - 1
\]

The fold-change in β-catenin is in general a function of all three groups of parameters, α, β, and γ. However, as shown in Figure 3B, there is a region of parameters where the fold-change in β-catenin is insensitive to variation in the dimensionless groups α and γ. This region is located where α >> 1 and γ << 1. Applying these conditions reduces eqn. 38 to:

\[
\frac{dx_{11}}{d\tau} \approx \alpha - \frac{\alpha}{1 + \delta} \cdot x_{11}
\]

and the Wnt-induced fold-change in β-catenin becomes independent of α and γ,

\[
F = 1 + \delta
\]

(the same result is obtained if we apply the conditions α >> 1 and γ << 1 directly to eqn. 30).

Within the insensitive region of parameters, two terms drop out from eqn. 38:

1. The DC-independent degradation
2. The positive feedback term (the APC-β-catenin binding)

and eqn. 38 reduces to a simple balance of production and degradation.

The fold-change in β-catenin is insensitive to variation in most parameters within the pathway, and a function of the ligand-containing terms only, if the DC-dependent degradation proceeds very rapidly (by more than 10-fold faster than other processes in the pathway). This is by no means trivial since it requires a
very rapid cycle of binding, phosphorylation, and dissociation. As a result, the DC-dependent degradation dominates over other routes for degrading β-catenin and renders the positive feedback interaction negligible.
Figure S1. Other Features of β-Catenin Accumulation Are Sensitive to Parameters

The Xenopus parameters were used as the “unperturbed system”. They were increased by 5-fold, one at a time. For each set of parameters, we simulated β-catenin accumulation in response to Wnt, and recorded the different features of β-catenin accumulation.
Figure S2. The Insensitivity to Parameters Can Still Be Seen when the Total Pool of $\beta$-Catenin (the Measurable Quantity) Is Examined Instead

Same as Figure S1, except that the total pool of $\beta$-catenin is plotted here, as opposed to free, un-phosphorylated $\beta$-catenin in Figure S1. The total pool of $\beta$-catenin is the quantity that can be measured using Western blot.
As a control, the basal level of β-catenin is not affected by variations in δ (~ligand stimulation). The Wnt-induced level and fold-change in β-catenin is sensitive to δ. The higher γ (~higher Wnt concentration), the higher the Wnt-induced level of β-catenin, and consequently the fold-change in β-catenin. γ=1 is used in these calculations.
RKO cells were pre-treated with different doses of LiCl (0-80mM) for 3h (in growth media). After that, the cells were transferred into control media or Wnt3A-conditioned media (WCM) + 0-80mM lithium. Cells were harvested usually at 24h, lysed, and analyzed using dot blot.

\( \beta\text{-catenin (-Wnt)} \) = level of \( \beta\text{-catenin} \) in cells incubated in control media + 0-80mM lithium. This normally changed little from 0h onward (i.e., the effects of lithium equilibrated rapidly).

\( \beta\text{-catenin (+Wnt)} \) = level of \( \beta\text{-catenin} \) in cells incubated in WCM + 0-80mM lithium after the new plateau in \( \beta\text{-catenin} \) was reached. The time to reach the new Wnt-induced plateau varied from ~3h (in untreated cells) to ~20-24h (in cells treated with the highest dose of lithium). It was most efficient to harvest all cells at the same time and it gave the same result as when the cells were harvested as soon as \( \beta\text{-catenin} \) reached the new plateau.

**Wnt-induced fold-change in \( \beta\text{-catenin} \) =** \( \beta\text{-catenin (+Wnt)} / \beta\text{-catenin (-Wnt)} \)

Control media: Using DMEM+serum or L-cell-conditioned media gave the same results. L cell line is the parental line for the L-Wnt3A cell line. Pre-treatment period: 3h or 6h pre-treatment gave the same result. The same protocol was used for BIO treatment.
RKO cells treated with different doses of lithium showed a dose-dependent increase in the level of β-catenin. Level of β-catenin was quantified using dot blot. The measurements reported here were taken after 24h incubation, but the same results were obtained from measurements at various time points (6h, 12h, 24h).
Cells were pre-incubated with BIO for 3 hours, followed by Wnt treatment in the presence of BIO for 6-24 hours. The level of β-catenin was quantified using dot blot. As a negative control, treatment with DMSO or MeBIO gave no significant changes in the level of β-catenin.
Figure S7. More on Inhibiting GSK3β in Cells Overexpressing β-Catenin

These measurements were performed in the same experiment, side by side. For each data point, 2 biological replicates were examined; SD is the std. dev. from 3 independent dot blots. In the main text, these data are included in Figures 4E-G. Here, they are presented side by side to show more directly that:

1) Cells overexpressing β-catenin responded more to Wnt stimulation (i.e., gave a higher fold-change).
2) Cells overexpressing β-catenin were less buffered when treated with lithium (i.e., fold-change decreased more readily).

More repeat experiments are shown in the main text.
To illustrate how Figure 5A was generated, let us decrease $\alpha$ from the *Xenopus* setpoint. As depicted by the green arrow in Figure A, the basal level of $\beta$-catenin will increase whereas the Wnt-induced fold-change in $\beta$-catenin will remain constant initially. As shown in Figure B, we then plot independently $\beta$-catenin level and fold-change as a function of $\alpha$. Figure C merges the two plots in Figure B. For each value of $\alpha$, we plot fold-change against basal level.
Figure S9. More Protocol Details on Experiments in the *Xenopus laevis* Embryos
To separate the cadherin-bound pool of β-catenin, a standard protocol was followed (Guger and Gumbiner, 2000; McCrea et al., 1991). 100 uL of embryo lysates (10 embryos) were rotated at 4°C for 1h with 100uL Concanavalin A–Sepharose 4B slurry (Sigma C9017). Supernatant and beads were separated by centrifugation (three 5min spin at 16,000g).

The supernatant showed trace bands with monoclonal anti-cadherin 6B6 (Hybridoma Bank, 1:500). The 6B6 detects C-/U-/EP-cadherin, the cadherins expressed in early embryos (Heasman et al., 1994). The lower bands (<100kDa) are likely to be cadherin degradation products because they disappear with RIPA-extraction.

Calibrating the volume of ConA to use. Equal volumes were loaded for each lane. Ratio does not necessarily correspond to actual membrane/cytoplasmic ratio of β-catenin in the embryo.
Figure S11. The Phenotypic Buffering in Response to \( \leq 200 \) mM Lithium Treatment Is Highly Reproducible

30-130 embryos were scored / data point shown.
Figure S12. Quantitation of β-Catenin at Earlier Stages Does Not Alter Conclusions

LiCl treatment

![Graph showing LiCl treatment effects](image)

β-catenin mRNA injection

![Graph showing β-catenin mRNA injection effects](image)

At all stages measured, β-catenin mRNA injection has lower effect on β-catenin protein level, and yet gives more severe phenotypes.
Figure S13. Transcription Is Not Saturated

And can be increased further with stronger lithium treatment

Expression of target genes eventually increases
Table S1. Complete Data from Experiments in *Xenopus laevis*

Controls are sibling embryos from the same mother and fertilization. Amount of RNA is a rough estimate. To get a dose-response, the same RNA stock solution was diluted in different volumes of nuclease-free water. In most experiments, all embryos were photographed and re-scored blindly on different days (the photos are available upon request); the results were similar. Different experiments were days to weeks apart; multiple females were used in an experiment.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Lithium treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of embryos scored</td>
<td>Median DAI (10th, 90th percentile)</td>
</tr>
<tr>
<td>Exp 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>109</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>150 mM</td>
<td>106</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>300 mM</td>
<td>104</td>
<td>6 (5, 7)</td>
</tr>
<tr>
<td>Exp 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>75 mM</td>
<td>40</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>200 mM</td>
<td>60</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>300 mM</td>
<td>76</td>
<td>5 (5, 6)</td>
</tr>
<tr>
<td>Exp 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>17 pg</td>
<td>33</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>Control</td>
<td>153</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>35 pg</td>
<td>93</td>
<td>5 (5, 5.2)</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
<td>5 (5, 5)</td>
</tr>
<tr>
<td>70 pg</td>
<td>45</td>
<td>5 (5, 6)</td>
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GBP mRNA injection
<table>
<thead>
<tr>
<th>Exp.</th>
<th># of embryos scored</th>
<th>Median DAI (10th, 90th percentile)</th>
<th>Changes in overall level of β-cat (SD)</th>
<th># of measurements (Western blot)</th>
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<tbody>
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<td>Exp 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>19</td>
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<tr>
<td>140 pg</td>
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<td>2.7 ± 0.1</td>
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<tr>
<td></td>
<td><strong>Axin1 mRNA injection</strong></td>
<td></td>
<td></td>
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<td>Exp 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>25 pg</td>
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<tr>
<td>Control</td>
<td>89</td>
<td>5 (5, 5)</td>
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</tr>
<tr>
<td>100 pg</td>
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<td>0.5 ± 0.1</td>
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</tr>
<tr>
<td>Exp 3</td>
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<td></td>
<td></td>
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<td>Control</td>
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<tr>
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<td>36</td>
<td>5 (5, 5)</td>
<td>0.73 ± 0.03</td>
<td>3</td>
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<td>Exp 1</td>
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<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>107</td>
<td>5 (5, 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;250 pg</td>
<td>17</td>
<td>3 (2, 4)</td>
<td></td>
<td></td>
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<td></td>
<td><strong>β-catenin mRNA injection</strong></td>
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<td>92</td>
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<td></td>
<td>1</td>
</tr>
<tr>
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<td>92</td>
<td>5 (5, 5)</td>
<td>1.0 ± 0.1</td>
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<tr>
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<td>56</td>
<td>5 (5, 5)</td>
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<td>1</td>
</tr>
<tr>
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<td>46</td>
<td>6 (5, 7)</td>
<td>1.5 ± 0.2</td>
<td>2</td>
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<tr>
<td>Control</td>
<td>54</td>
<td>5 (5, 5)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>125 pg</td>
<td>72</td>
<td>5 (5, 6)</td>
<td>1.6 ± 0.2</td>
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</tr>
<tr>
<td>Exp.</td>
<td># of embryos scored</td>
<td>Median DAI (10th, 90th percentile)</td>
<td>Changes in overall level of β-cat (SD)</td>
<td># of measurements (Western blot)</td>
</tr>
<tr>
<td>------------</td>
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<td><strong>Exp 15</strong></td>
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<tr>
<td>Control</td>
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<td>5 (5, 5)</td>
<td></td>
<td>1</td>
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<tr>
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<td>5 (5, 6)</td>
<td>1.1 ± 0.3</td>
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<tr>
<td>Control</td>
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<td></td>
<td>1</td>
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<tr>
<td>125 pg – M1920</td>
<td>25</td>
<td>6 (5, 8)</td>
<td>1.4</td>
<td></td>
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<td><strong>Exp 13</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>5 (4, 5)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>125 pg</td>
<td>14</td>
<td>6 (5, 7)</td>
<td>1.4 ± 0.1</td>
<td>4</td>
</tr>
<tr>
<td>125 pg – 2cell st.</td>
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<td>6 (5, 7)</td>
<td>1.5 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>250 pg</td>
<td>12</td>
<td>7 (5.7, 8)</td>
<td>2.3 ± 0.1</td>
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</tbody>
</table>
**Negative control: Bias due to expectation to see effects**

In these experiments, we injected trace amount of $\beta$-catenin mRNA without realizing it was a trace amount (a 10-fold mistake in dilution). The mistake was not discovered until we performed scoring and Western blots. The result was a negative control that allowed us to examine the expectation bias in scoring and measurements.

<table>
<thead>
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<th>Exp 10</th>
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<tbody>
<tr>
<td>Control</td>
<td>54</td>
<td>5 (5, 5)</td>
<td>1</td>
</tr>
<tr>
<td>10 pg</td>
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<td>1.2 ± 0.3</td>
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</tr>
<tr>
<td>10 pg</td>
<td>18</td>
<td>1.1 ± 0.5</td>
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<table>
<thead>
<tr>
<th>Exp 11</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>47</td>
<td>5 (5, 5)</td>
<td>1</td>
</tr>
<tr>
<td>14 pg</td>
<td>30</td>
<td>1.2 ± 0.1</td>
<td>2</td>
</tr>
<tr>
<td>20 pg</td>
<td>27</td>
<td>0.9 ± 0.5</td>
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### Table S2. RT-PCR FAM-Probe/Primers Sequences

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<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Probe</th>
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</thead>
<tbody>
<tr>
<td>siamois</td>
<td>5’-CAGTCAACAGGACTTTAAAAGG-3’</td>
<td>5’-GTACTGGTGCTGGCTGAGAAAT-3’</td>
<td>5’-GAAGAT<em>GAACA</em>T*AGAG-3’</td>
</tr>
<tr>
<td>Xnr3</td>
<td>5’-CCATTCCTACTTAAGAGTGTG-3’</td>
<td>5’-CTCTTCATGGTGCCCTCAGGATA-3'</td>
<td>5’-GTGTCCCTCCTGTGTGC-3’</td>
</tr>
<tr>
<td>ODC</td>
<td>5’-CCCGAGCGGGATTATCTAT-3’</td>
<td>5’-GGGTGATTCCTTGCCACCTTT-3’</td>
<td>5’-ACAAGTTTCCAGAT*CA-3’</td>
</tr>
</tbody>
</table>

Grey highlights: predicted exon-exon boundaries
* indicates superbase analog.
Primer/probe sets were designed using the Qiagen primer designer.


