Network description as set of ODEs

In the set of ODEs, binding and dissociation processes are described by the rate equations: $k_i \cdot X \cdot Y - k_{-i} \cdot (X/Y)$ where X and Y denote the free concentrations of the binding partners, (X/Y) the concentration of the complex, and k_i and k_{-i} the association and dissociation rates respectively of the complexes formed by the proteins (i denotes the reaction number as specified in Figure 2, main text). Syntheses of proteins are described by constant rates (v_i). Phosphorylation and dephosphorylation procesess are described by linear rate equations $(k_i \cdot X)$. All rate constants along with their sources are included in Table S2, which is published as supporting information.

Variables

Molecules in system

X_1 - Wnt3a
X_2 - (Wnt3a/LRP^)
X_3 - (Wnt3a/LRP^/APC/Axin/GSK3)
X_4 - (APC/Axin/GSK3)
X_5 - (APC*/Axin*/GSK3)
X_6 - (Axin/GSK3)
X_7 - APC
X_8 - (β-cat/APC)
X_9 - (β-cat/APC*/Axin*/GSK3)
X_{10} - β-cat
X_{11} - TCF
X_{12} - (β-cat/TCF)
X_{13} – *DPAGT1* mRNA
X_{14} – GPT
X_{15} - LRP
X_{16} - LRP^

X_{17} – $(\text{E-cad/ β-cat})_{ER}$	- ER = Endoplasmic Reticulum
X_{18} – $(\text{E-cad/ β-cat})_{M}$	- M = Membrane
X_{19} – $(\text{E-cad/ β-cat})_{ERC}$	- ERC = Endocytic Recycling Compartment
X_{20} – AJ	- AJ = Adherens Junctions

Adhesivity factor of E-cadherin based on extent of N-glycosylation in different pools

σ_{ER} - Adhesivity of E-cadherin in ER
σ_{M} - Adhesivity of E-cadherin in M
σ_{ERC} - Adhesivity of E-cadherin in ERC
σ_{AJ} - Adhesivity of E-cadherin in AJ

Transcriptional activation by a single activator (i.e. (β-cat/TCF) or X_{12}) is modeled as a Hill-type activation. This has been demonstrated to be appropriate for this system, recreating published experimental data (1, 2). This is shown in Equation 1:

$$\varphi(X_{12}, K_{TmRNA}, \nu) = \frac{X_{12}^{\nu}}{(K_{TmRNA}^{\nu} + X_{12}^{\nu})} \quad \textbf{[1]}$$

where K_{TmRNA} is the activator concentration at which transcription proceeds at half of its maximal rate and ν describes the degree of nonlinearity in the activation (cooperativity or Hill coefficient). Subsequent translation of the resulting *DPAGT1* mRNA is modeled with enzyme synthesis being determined by the amount of mRNA (X_{13}) and a maximum rate of translation (P_{max}). Equation 2 describes their relation:

$$\chi(X_{13}, P_{max}) = P_{max} X_{13} \quad \textbf{[2]}$$

For the N-glycosylation of LRP (X_{15}), it is assumed that binding of GPT (X_{14}) and LRP is non-reversible and conversion into N-glycosylated LRP or LRP^ (X_{16}) occurs much faster than the binding between substrate and enzyme. This results in Equation 3:

$$\psi(X_{14}, X_{15}, k_{17}) = k_{17} X_{14} X_{15} \quad \textbf{[3]}$$

Enzymatic activity of GPT on E-cadherin was modeled differently, because the extent of N-glycosylation of E-cadherin modulates homotypic binding of E-cadherin rather than determine whether it will be transported to the membrane or not (as with LRP5/6). To model this effect an adhesivity factor was introduced (σ): This value is normalized to the maximum concentration of N-glycosylated E-cadherin that can be synthesized in a single step (heuristically determined); when σ = 0, the E-cadherin is completely non-adhesive, and when σ = 1, it is the most adhesive. This time varying factor is calculated for each of the four pools included in the reaction scheme: endoplasmic reticulum (ER), membrane (M), endocytic recycling compartment (ERC), and adherens junctions (AJ). The change over time in E-cadherin adhesivity for each pool is calculated based on the fraction of incoming E-cadherin to the new total concentration of E-cadherin in the pool (f_{gain}) and the adhesivity of the incoming and receiving E-cadherin, σ_{source} and $\sigma_{destination}$ respectively. This change is described by Equation 4:

$$\frac{d\sigma_{destination}}{dt} = (f_{gain})(\sigma_{source} - \sigma_{destination}) \quad \textbf{[4]}$$

Because E-cadherin in the ER pool is synthesized and not transported from a source pool, σ_{ER} is dependent on the concentration of GPT at the time of synthesis. The rate at which E-cadherin in the ER is N-glycosylated is calculated assuming Michaelis-Menten kinetics of GPT with no cooperativity.

Starting with the Michaelis-Menten equation (Equation 5):

$$v = \frac{V_{max}[S]}{K_M + [S]} \quad \textbf{[5]}$$

Because the substrate to GPT is lipid-linked oligosaccharide (LLO) and cells are not being modeled under a shortage of carbohydrates, it is assumed that it is the availability of enzyme and not the substrate what determines the kinetics of the reaction. In this case, to adapt Michaelis-Menten kinetics to this system, two assumptions are made: First, a quasi-steady state of the substrate (*i.e.* the concentration of the substrate/product changes much more slowly than that of the enzyme). Second, the substrate (*i.e.* N-glycans) is unlimited.

We multiply both sides of Equation 5 by time (t) to get the amount of product on the LHS and divide by G_{max} (the maximum possible concentration of N-glycosylated E-cadherin in the ER) to normalize the amount of product. Because E-cadherin is more adhesive when extent of N-glycosylation is low, and *vice versa*, the ratio of product is subtracted from 1 to define σ at the time of synthesis (Equation 6):

$$\sigma_{synthesis} = 1 - \frac{vt}{G_{max}} = 1 - \frac{t}{G_{max}} \frac{V_{max}[GPT]}{K_M + [GPT]} \quad \textbf{[6]}$$

Thus the change in σ_{ER} over time is given by Equation 7:

$$\frac{d\sigma_{ER}}{dt} = (f_{gain})[(1 - \frac{V_{max} t}{G_{max}} \frac{X_{14}}{K_M + X_{14}}) - \sigma_{ER}] \quad \textbf{[7]}$$

V_{max} represents the maximum rate of N-glycosylation of E-cadherin by GPT in the ER. K_M represents the GPT concentration at which enzymatic activity is half-maximal.

To describe the dependence of the rate of AJ formation (k_{24}) on σ_M, a simple linear relation was chosen due to the difficulty in defining this relation experimentally as cellular environment is believed to be highly variable and influential to this relation. Similarly, an inverse linear relation was chosen for the dependence of the rate of AJ dissociation (k_{-24}) on σ_{AJ}. Equations 8 and 9 describe these relations:

$$k_{24}(\sigma_M) = k_{aj}\, \sigma_M \quad \textbf{[8]}$$

$$k_{-24}(\sigma_{AJ}) = -k_{daj}\,\sigma_{AJ} + k_{Mdaj} \quad \textbf{[9]}$$

k_{aj} represents the fastest possible rate of AJ formation (when $\sigma_M = 1$), k_{daj} is the drop in rate of AJ disruption when going from minimal to maximal E-cadherin adhesivity, and k_{Mdaj} is the fastest possible rate of AJ disruption (set equal to k_{daj} in model for reported results).

System of ODEs

$$\frac{dX_1}{dt} = k_{-1}X_2 - k_1X_1X_{16}$$

$$\frac{dX_2}{dt} = k_1X_1X_{16} - k_{-1}X_2 + k_{-2}X_3 - k_2X_2X_4$$

$$\frac{dX_3}{dt} = k_2X_2X_4 - k_{-2}X_3$$

$$\frac{dX_4}{dt} = k_{-2}X_3 - k_2X_2X_4 - k_4X_4 + k_5X_5 + k_3X_6X_7 - k_{-3}X_4$$

$$\frac{dX_5}{dt} = k_4X_4 - k_5X_5 + k_7X_9 + k_{-6}X_9 - k_6X_5X_{10}$$

$$\frac{dX_6}{dt} = k_{-3}X_4 - k_3X_6X_7$$

$$\frac{dX_7}{dt} = k_{-3}X_4 - k_3X_6X_7 + k_{-8}X_8 - k_8X_7X_{10}$$

$$\frac{dX_8}{dt} = k_8X_7X_{10} - k_{-8}X_8$$

$$\frac{dX_9}{dt} = k_6X_5X_{10} - k_{-6}X_9 - k_7X_9$$

$$\frac{dX_{10}}{dt} = \nu_9 + k_{-6}X_9 - k_6X_5X_{10} + k_{-8}X_8 - k_8X_7X_{10} + k_{-11}X_{12} - k_{11}X_{10}X_{11} + k_{25}X_{19} - k_{10}$$

$$\frac{dX_{11}}{dt} = k_{-11}X_{12} - k_{11}X_{10}X_{11}$$

$$\frac{dX_{12}}{dt} = k_{11}X_{10}X_{11} - k_{-11}X_{12}$$

$$\frac{dX_{13}}{dt} = T_{max}\left[\frac{X_{12}^{\nu}}{(K_{TmRNA}^{\nu} + X_{12}^{\nu})}\right] - k_{13}\,X_{13}$$

$$\frac{dX_{14}}{dt} = P_{max}\,X_{13} - k_{19}\,X_{14}$$

$$\frac{dX_{15}}{dt} = \nu_{15} - k_{17}\,X_{14}\,X_{15} - k_{16}X_{15}$$

$$\frac{dX_{16}}{dt} = k_{17}X_{14}\,X_{15} - k_{18}X_{16} + k_{-1}X_2 - k_1X_1X_{16}$$

$$\frac{dX_{17}}{dt} = v_{20} - k_{21}X_{17}$$

$$\frac{dX_{18}}{dt} = k_{21}X_{17} - k_{22}X_{18} + k_{23}X_{19} + k_{-24}(\sigma_{AJ})X_{20} - k_{24}(\sigma_M)X_{20}$$

$$\frac{dX_{19}}{dt} = k_{22}X_{18} - k_{23}X_{19} - k_{25}X_{19} - k_{26}X_{19}$$

$$\frac{dX_{20}}{dt} = k_{24}(\sigma_M)X_{18} - k_{-24}(\sigma_{AJ})X_{20}$$

$$\frac{d\sigma_{ER}}{dt} = \left(\frac{v_{20}}{X_{17}}\right)\left[\left(1 - \frac{V_{max}t}{G_{max}}\frac{X_{14}}{K_M + X_{14}}\right) - \sigma_{ER}\right]$$

$$\frac{d\sigma_M}{dt} = \left(\frac{k_{21}X_{17}}{X_{18}}\right)(\sigma_{ER} - \sigma_M) + \left(\frac{k_{23}X_{19}}{X_{18}}\right)(\sigma_{ERC} - \sigma_M) + \left(\frac{k_{-24}(\sigma_{AJ})X_{20}}{X_{18}}\right)(\sigma_{AJ} - \sigma_M)$$

$$\frac{d\sigma_{ERC}}{dt} = \left(\frac{k_{22}X_{18}}{X_{19}}\right)(\sigma_M - \sigma_{ERC})$$

$$\frac{d\sigma_{AJ}}{dt} = \left(\frac{k_{24}(\sigma_M)X_{18}}{X_{20}}\right)(\sigma_M - \sigma_{AJ})$$

From ODEs to DAEs

Rapid Equilibrium Approximation:

A fast equilibrium approximation was used for reactions *i* = 1, 2, 6, 8. Their equilibrium constants can be described algebraically by Equation 10:

$$K_i = \frac{k_{-i}}{k_i} = \frac{X \cdot Y}{(X/Y)} \quad \textbf{[10]}$$

The resulting equations are shown as Equations 11-15:

$$X_2 = \frac{X_1 X_{16}}{K_1} \quad \textbf{[11]}$$

$$X_3 = \frac{X_2 X_4}{K_2} \quad \textbf{[12]}$$

$$X_9 = \frac{X_5 X_{10}}{K_6} \quad \textbf{[13]}$$

$$X_8 = \frac{X_7 X_{10}}{K_8} \quad \textbf{[14]}$$

$$X_{12} = \frac{X_{10} X_{11}}{K_{11}} \quad \textbf{[15]}$$

The individual forward and backward rates, k_i and k_{-i}, were removed from the ODEs by linearly combining equations with these rate constants, reducing the number of parameters needed to describe these reactions from two to one. More specifically, the following linear combinations of ODEs were performed:

$$-\frac{dX_1}{dt}+\frac{dX_3}{dt}+\frac{dX_4}{dt}+\frac{dX_{16}}{dt}=\cdots \quad \textbf{[16]}$$

$$-\frac{dX_5}{dt}+\frac{dX_8}{dt}+\frac{dX_{10}}{dt}+\frac{dX_{12}}{dt}=\cdots \quad \textbf{[17]}$$

$$\frac{dX_5}{dt}+\frac{dX_9}{dt}=\cdots \quad \textbf{[18]}$$

$$\frac{dX_7}{dt}+\frac{dX_8}{dt}=\cdots \quad \textbf{[19]}$$

Conservation equations:

Another set of algebraic equations came from the conservation of molecules observed to be expressed constitutively in cells. These molecules were Wnt3a, APC, TCF, and (Axin/GSK3). Their total concentration was represented by the parameters WNT^0, APC^0, TCF^0, and $(Axin/GSK3)^0$, respectively. The resulting conservation equations were:

$$X_1+X_2+X_3=WNT^0 \quad \textbf{[20]}$$

$$X_3+X_4+X_5+X_7+X_8+X_9=APC^0 \quad \textbf{[21]}$$

$$X_3+X_4+X_5+X_6+X_9=(Axin/GSK3)^0 \quad \textbf{[22]}$$

$$X_{11}+X_{12}=TCF^0 \quad \textbf{[23]}$$

Given that $APC^0 >> (Axin/GSK3)^0$, Equation 21 was simplified to Equation 24:

$$X_7+X_8=APC^0 \quad \textbf{[24]}$$

System of DAEs

Independent variables: X_4, X_5, X_{10}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, σ_{ER}, σ_M, σ_{ERC}, σ_{AJ}
Dependent variables: X_1, X_2, X_3, X_6, X_7, X_8, X_9, X_{11}, X_{12}

Algebraic equations:

$$X_1=\frac{WNT^0K_1K_2}{K_1K_2+K_2X_{16}+X_4X_{16}}$$

$$X_2=\frac{WNT^0K_2X_{16}}{K_1K_2+K_2X_{16}+X_4X_{16}}$$

$$X_3=\frac{WNT^0X_4X_{16}}{K_1K_2+K_2X_{16}+X_4X_{16}}$$

$$X_6=(Axin/GSK3)^0-\left(1+\frac{WNT^0X_{16}}{K_1K_2+(K_2+X_4)X_{16}}\right)X_4-(1+\frac{X_{10}}{K_6})X_5$$

$$X_7 = \frac{APC^0}{1 + \frac{X_{10}}{K_8}}$$

$$X_8 = \frac{X_7\, X_{10}}{K_8}$$

$$X_9 = \frac{X_5\, X_{10}}{K_6}$$

$$X_{11} = \frac{TCF^0 K_{11}}{K_{11} + X_{10}}$$

$$X_{12} = \frac{TCF^0 X_{10}}{K_{11} + X_{10}}$$

Differential equations:

$$\frac{dX_6}{dt} = k_{-3}X_4 \; - \; k_3 X_6 X_7$$

$$\frac{dX_{13}}{dt} = \; T_{max}\,[\,\frac{X_{12}^{\nu}}{(K_{TmRNA}^{\nu} + \; X_{12}^{\nu})}\,] \; - \; k_{13}\, X_{13}$$

$$\frac{dX_{14}}{dt} = \; P_{max}\, X_{13} \; - \; k_{19}\, X_{14}$$

$$\frac{dX_{15}}{dt} = \; v_{15} \; - \; k_{17}\, X_{14}\, X_{15} - k_{16} X_{15}$$

$$\frac{dX_{17}}{dt} = \; v_{20} \; - \; k_{21} X_{17}$$

$$\frac{dX_{18}}{dt} = \; k_{21}\, X_{17} - \; k_{22} X_{18} + \; k_{23} X_{19} + \; k_{-24}(\sigma_{AJ})\, X_{20} - \; k_{24}(\sigma_M)\, X_{18}$$

$$\frac{dX_{19}}{dt} = \; k_{22} X_{18} - \; k_{23} X_{19} - \; k_{25} X_{19} - \; k_{26} X_{19}$$

$$\frac{dX_{20}}{dt} = \; k_{24}(\sigma_M) X_{18} - k_{-24}(\sigma_{AJ})\, X_{20}$$

$$\frac{d\sigma_{ER}}{dt} = \left(\frac{v_{20}}{X_{17}}\right)[(1 - \frac{V_{max} t}{G_{max}} \frac{X_{14}}{K_M + X_{14}}) - \sigma_{ER}]$$

$$\frac{d\sigma_M}{dt} = \; \left(\frac{k_{21} X_{17}}{X_{18}}\right)(\sigma_{ER} - \sigma_M) + \; \left(\frac{k_{23} X_{19}}{X_{18}}\right)(\sigma_{ERC} - \sigma_M) + \; (\frac{k_{-24}(\sigma_{AJ}) X_{20}}{X_{18}})(\sigma_{AJ} - \sigma_M)$$

$$\frac{d\sigma_{ERC}}{dt} = \; \left(\frac{k_{22} X_{18}}{X_{19}}\right)(\sigma_M - \sigma_{ERC})$$

$$\frac{d\sigma_{AJ}}{dt} = \left(\frac{k_{24}(\sigma_M)X_{18}}{X_{20}}\right)(\sigma_M - \sigma_{AJ})$$

Implicit differential equations:

$$-\frac{dX_5}{dt} + \frac{dX_{10}}{dt}\left[\frac{\delta X_8}{\delta X_{10}} + \frac{\delta X_{12}}{\delta X_{10}} + 1\right] = -k_4X_4 + k_5X_5 - k_7X_9 + \nu_9 + k_{25}X_{19} - k_{10}$$

$$\frac{dX_5}{dt}\left[\frac{\delta X_9}{\delta X_5} + 1\right] + \frac{dX_{10}}{dt}\left[\frac{\delta X_9}{\delta X_{10}}\right] = k_4X_4 - k_5X_5$$

$$\frac{dX_4}{dt}\left[-\frac{\delta X_1}{\delta X_4} + \frac{\delta X_3}{\delta X_4} + 1\right] + \frac{dX_{16}}{dt}\left[-\frac{\delta X_1}{\delta X_{16}} + \frac{\delta X_3}{\delta X_{16}} + 1\right] = k_{17}X_{14}X_{15} - k_{18}X_{16} - k_4X_4 + k_5X_5 + k_3X_6X_7 - k_{-3}X_4$$

REFERENCES

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2. Biechele TL, Moon RT (2008) Assaying beta-catenin/TCF transcription with beta-catenin/TCF transcription-based reporter constructs. *Methods Mol Biol* 468:99–110.

Parameter values

Parameter	Value	Units
WNT^0	0 (off), 28.062 (on)	nM
APC^0	100	nM
TCF^0	15	nM
$(Axin/GSK3)^0$	0.02	nM
β-cat^0	35 (off)	nM
K_1	6	nM
K_2	1/12	nM
k_4	0.26	min^{-1}
k_5	0.13	min^{-1}
k_3	0.091	$nM^{-1}\ min^{-1}$
k_{-3}	0.91	min^{-1}
K_6	100	nM
k_7	210	min^{-1}
K_8	10 K_6	nM
v_9	0.6	$nM\ min^{-1}$
k_{10}	0.00026	min^{-1}
K_{11}	30	nM
T_{max}	0.005946	$nM\ min^{-1}$
K_{TmRNA}	10	nM
v	3	
P_{max}	0.025	min^{-1}
k_{13}	0.11781	min^{-1}
v_{15}	0.1	$nM^{-1}\ min^{-1}$
k_{16}	0.02	min^{-1}
k_{17}	0.1	$nM^{-1}\ min^{-1}$
k_{18}	0.015	min^{-1}
k_{19}	0.0924	min^{-1}
v_{20}	0.000036	$nM\ min^{-1}$
k_{21}	1/60	min^{-1}
k_{22}	0.0123	min^{-1}
k_{23}	0.0005	min^{-}
k_{25}	0.02	min^{-1}
k_{26}	0.01	min^{-1}
$V_{max}t/G_{max}$	1.1	
K_M	0.00887	nM
k_{aj}	0.01	min^{-1}
k_{daj}	0.02	min^{-1}

Parameters are grouped in the following categories from top to bottom: constitutive protein concentrations, Wnt3a binding, active β-catenin regulation, *DPAGT1* expression, N-glycosylation, E-cadherin recycling, and AJ formation/dissociation.