

# Pattern formation by dynamically interacting network motifs

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## Description of the mathematical model

The model for the network in Figure 1C is given by the following equations:

$$\begin{aligned}
\frac{\partial[GRK]}{\partial T} &= D_{GRK} \frac{\partial^2[GRK]}{\partial X^2} - K_{d,GRK} \cdot [GRK] \cdot [EGFR] + V_{GRK} \cdot h^+(X_{GRK} - X) \\
\frac{\partial[DPP]}{\partial T} &= D_{DPP} \frac{\partial^2[DPP]}{\partial X^2} - K_{d,DPP} \cdot [DPP] \cdot [TKV] \\
\frac{\partial[PNT]}{\partial T} &= \gamma_{PNT} \cdot h^+([S_{EGFR}], \theta_{[S_{EGFR}], [PNT]}) - k_{d,PNT} \cdot [PNT] \\
\frac{\partial[TKV]}{\partial T} &= \gamma_{TKV} \cdot h^+([BR], \theta_{BR,TKV}) - k_{d,TKV} \cdot [TKV] - K_{d,DPP} \cdot [DPP] \cdot [TKV] \\
\frac{\partial[BRK]}{\partial T} &= \gamma_{BRK} \cdot h^+([S_{EGFR}], \theta_{[S_{EGFR}], [BRK]}) \cdot h^-([S_{DPP}], \theta_{[S_{DPP}], [BRK]}) - k_{d,BRK} \cdot [BRK] \\
\frac{\partial[br]}{\partial T} &= \gamma_{br} \cdot (h^+([S_{EGFR}], \theta_{[S_{EGFR}], [br]}) + f_{br,g.i.}) \cdot h^-([PNT], \theta_{[PNT], [br]}) \cdot \\
&\quad f_{rep}([S_{DPP}], [BRK], \theta_{[S_{DPP}], [br]}, \theta_{[BRK], [br]}) - k_{d,br} \cdot [br] \\
\frac{\partial[BR]}{\partial T} &= \gamma_{BR} \cdot [br] - k_{d,BR} \cdot [BR] \\
[S_{EGFR}] &= \alpha_{EGFR} \cdot \frac{k_{on,GRK}}{k_{off,GRK} + k_{e,GRK}} \cdot [GRK] \cdot [EGFR] \\
[S_{DPP}] &= \alpha_{DPP} \cdot \frac{k_{on,DPP} \cdot k_{e,DPP}}{(k_{off,DPP} + k_{e,DPP}) \cdot k_{d,C_{DPP-TKV}}} \cdot [DPP] \cdot [TKV]
\end{aligned} \tag{S1}$$

GRK is secreted from the dorsal anterior cortex of the oocyte. The production term in the rate equation for GRK has a value of  $V_{GRK}$  for  $X < X_{GRK}$  and 0 otherwise. Thus,  $X_{GRK}$  marks the most lateral point of GRK secretion domain. Since DPP enters the follicular epithelium as an anterior flux, its production is described in the anterior boundary condition instead of in the rate equation itself. Ligand degradation is due to receptor-mediated endocytosis.

$$\begin{aligned}
\underbrace{\frac{\partial[GRK]}{\partial T}}_{\text{rate of change of concentration}} &= \underbrace{D_{GRK} \frac{\partial^2[GRK]}{\partial X^2}}_{\text{diffusion}} - \underbrace{K_{d,GRK} \cdot [GRK] \cdot [EGFR]}_{\text{ligand-receptor interaction}} + \underbrace{V_{GRK} \cdot h^+(X_{GRK} - X)}_{\text{production}} \\
\underbrace{\frac{\partial[DPP]}{\partial T}}_{\text{rate of change of concentration}} &= \underbrace{D_{DPP} \frac{\partial^2[DPP]}{\partial X^2}}_{\text{diffusion}} - \underbrace{K_{d,DPP} \cdot [DPP] \cdot [TKV]}_{\text{ligand-receptor interaction}} \quad [S2]
\end{aligned}$$

The rate equations for other components in the model do not include a description for diffusion; only production and degradation terms are included in the right hand sides. PNT production is a function of its inductive signal: EGFR signaling. When the level of EGFR signaling  $[S_{EGFR}]$  is above the relevant threshold  $\theta_{[S_{EGFR}], [PNT]}$ , then the production function of PNT equals to  $\gamma_{PNT}$ . Otherwise, PNT production is equal to zero (Fig 2C). PNT degradation, similar to all other degradation terms, follows first order kinetics:

$$\underbrace{\frac{\partial[PNT]}{\partial T}}_{\text{rate of change of concentration}} = \underbrace{\gamma_{PNT} \cdot h^+([S_{EGFR}], \theta_{[S_{EGFR}], [PNT]})}_{\text{production}} - \underbrace{k_{d,PNT} \cdot [PNT]}_{\text{degradation}} \quad [S3]$$

The rate equation for TKV, the type I DPP receptor, involves not only production and degradation, but also the ligand-receptor interaction term:  $k_{d,DPP} \cdot [DPP] \cdot [TKV]$ . The production function of TKV depends on the level of BR in the similar fashion that PNT production depends on the level of EGFR signaling.

$$\underbrace{\frac{\partial[TKV]}{\partial T}}_{\text{rate of change of concentration}} = \underbrace{\gamma_{TKV} \cdot h^+([BR], \theta_{BR,TKV})}_{\text{production}} - \underbrace{k_{d,TKV} \cdot [TKV]}_{\text{degradation}} - \underbrace{K_{d,DPP} \cdot [DPP] \cdot [TKV]}_{\text{ligand-receptor interaction}} \quad [S4]$$

The production function of BRK relies on two quantities, the level of EGFR signaling and the level of DPP signaling. Thus, BRK is only produced (with production rate  $\gamma_{BRK}$ ) when both the level of EGFR signaling ( $[S_{EGFR}]$ ) is above the critical threshold  $\theta_{[S_{EGFR}], [BRK]}$  and the level of DPP signaling ( $[S_{DPP}]$ ) is below the critical threshold  $\theta_{[S_{DPP}], [BRK]}$ . Mathematically, BRK production is thus depicted by multiplication of its production dependence on EGFR signaling and its production dependence on DPP signaling. Such a mathematical depiction is chosen for simplicity. A more accurate description requires studies of the regulatory region of each target gene.

$$\underbrace{\frac{\partial[BRK]}{\partial T}}_{\text{rate of change of concentration}} = \underbrace{\gamma_{BRK} \cdot h^+([S_{EGFR}], \theta_{[S_{EGFR}], [BRK]}) \cdot h^-([S_{DPP}], \theta_{[S_{DPP}], [BRK]})}_{\text{production}} - \underbrace{k_{d,BRK} \cdot [BRK]}_{\text{degradation}} \quad [S5]$$

Similar to the production function of BRK, the production function of *br* also depends on the expression levels of multiple network components. *br* is only expressed when all

three conditions are met: 1. EGFR signaling must be higher than  $\theta_{[S_{EGFR}],[br]}$ , 2. PNT concentration must be lower than  $\theta_{[PNT],[br]}$ , and 3. DPP signaling must be lower than  $\theta_{[S_{DPP}],[br]}$  or BRK concentration has to be higher than  $\theta_{[BRK],[br]}$ .

$$\underbrace{\frac{\partial[br]}{\partial T}}_{\text{rate of change of concentration}} = \underbrace{\gamma_{br} \cdot (h^+([S_{EGFR}], \theta_{[S_{EGFR}],[br]}) + f_{br,g.i.}) \cdot h^-([PNT], \theta_{[PNT],[br]})}_{\text{production}} - \underbrace{k_{d,br} \cdot [br]}_{\text{degradation}}$$

$$f_{rep}([S_{DPP}], [BRK], \theta_{[S_{DPP}],[br]}, \theta_{[BRK],[br]}) = \begin{cases} 0 & \text{when } [S_{DPP}] > \theta_{[S_{DPP}],[br]} \ \& \ [BRK] < \theta_{[BRK],[br]} \\ 1 & \text{otherwise} \end{cases} \quad [S6]$$

For every other network component, we lumped the transcript and the protein into one species due to the lack of expression data of the proteins. However, BR antibody is available and the distribution of BR throughout oogenesis can be monitored. Although for most of oogenesis, BR expression mirrors the expression of its transcript, BR is still expressed in late oogenesis when the transcript is undetected, suggesting that BR is a more stable molecule compared to its transcript. Since there is a difference in the expressions data between the transcript and the protein, we have included BR as a separate species in the model.

BR is produced by translation of its transcript  $br$ . We assumed that the production of BR linearly depends on the concentration of  $br$ .

$$\underbrace{\frac{\partial[BR]}{\partial T}}_{\text{rate of change of concentration}} = \underbrace{\gamma_{BR} \cdot [br]}_{\text{production}} - \underbrace{k_{d,BR} \cdot [BR]}_{\text{degradation}} \quad [S7]$$

We assume that the levels of both GRK and DPP signaling,  $[S_{EGFR}]$  and  $[S_{DPP}]$ , are proportional to the occupancy of their receptors (for DPP signaling, it is proportional to the level of internalized ligand- receptor complexes (Fig 2B)):

$$[S_{EGFR}] = \alpha_{EGFR} \cdot [C_{GRK-EGFR}], \quad [S_{DPP}] = \alpha_{DPP} \cdot [C_{DPP-TKV}]_i \quad [S8]$$

where  $[C_{GRK-EGFR}]$  is the concentration of GRK-EGFR complexes and  $[C_{DPP-TKV}]_i$  is the concentration of internalized DPP-TKV complexes. The proportionality constants  $\alpha_{EGFR}$  and  $\alpha_{DPP}$  describe the combined effects of the EGFR and DPP pathway components downstream of activated receptors. Their values are equal to one in the wild type background, but are varied in mutants with defects in pathway activation. For example,  $\alpha_{EGFR} < 1$  in the *Ras* hypomorph mutant and  $\alpha_{DPP} = 0$  in the cells that overexpress *daughters against dpp* (*dad*), a gene encoding an intracellular inhibitor of DPP signaling.

Receptor occupancies are calculated based on the steady-state approximation for ligand-receptor kinetics (Fig 2B, the derivations can be found in our previous work and the description of parameters can be found in Table S2, (1, 2)):

$$\begin{aligned} [C_{GRK-EGFR}] &= \frac{k_{on,GRK}}{k_{off,GRK} + k_{e,GRK}} \cdot [GRK] \cdot [EGFR] \\ [C_{DPP-TKV}]_i &= \frac{k_{on,DPP} \cdot k_{e,DPP}}{(k_{off,DPP} + k_{e,DPP}) \cdot k_{d,C_{DPP-TKV}}} \cdot [DPP] \cdot [TKV] \end{aligned} \quad [S9]$$

Equations S1 above were then solved with the following boundary conditions:

$$\left. \frac{\partial [GRK]}{\partial X} \right|_{X=0} = \left. \frac{\partial [GRK]}{\partial X} \right|_{X=L} = 0, \quad \left. \frac{\partial [DPP]}{\partial X} \right|_{X=0} = V_{DPP}, \quad \left. \frac{\partial [DPP]}{\partial X} \right|_{X=L} = 0 \quad [S10]$$

where DPP enters the system from the anterior boundary as a constant flux  $V_{DPP}$  and both ligand concentrations are assumed to have leveled off at the posterior boundary ( $\partial [GRK] / \partial X|_{X=L} = \partial [DPP] / \partial X|_{X=L} = 0$ ). The GRK source term is already included in its rate equation, thus we imposed a no flux boundary condition for GRK at the anterior boundary  $X = 0$ .

Equations S1 were solved with the following initial conditions (see Table S2):

$$\begin{aligned} [GRK]_0 &= [DPP]_0 = [PNT]_0 = 0 \\ [br]_0 &= \alpha_{br}, \quad \alpha_{br} > 0 \\ [BR]_0 &= \gamma_{BR} \cdot \alpha_{br} \\ [TKV]_0 &= \alpha_{TKV}, \quad \alpha_{TKV} > 0 \\ [BRK]_0 &= \alpha_{BRK}, \quad \alpha_{BRK} > 0 \end{aligned} \quad [S11]$$

### Nondimensionalization of model equations

To reduce the number of free parameters, these equations were nondimensionalized as the following (the resulting nondimensionalized concentrations are scaled to range from zero to one):

$$\begin{aligned} GRK &= [GRK] / (V_{GRK} / k_{d,GRK}) & br &= [br] / (\gamma_{br} / k_{d,br}) \\ DPP &= [DPP] / (V_{DPP} \cdot L / D_{DPP}) & BR &= \frac{[Br]}{(\gamma_{BR} \cdot k_{d,BR} \cdot \gamma_{br} / k_{d,br})} \\ PNT &= [PNT] / (\gamma_{PNT} / k_{d,PNT}) & t &= T \cdot k_{d,br} \\ TKV &= [TKV] / (\gamma_{TKV} / k_{d,TKV}) & x &= X / L \\ BRK &= [BRK] / (\gamma_{BRK} / k_{d,BRK}) \end{aligned} \quad [S12]$$

In addition, we lumped the parameters into dimensionless groups (see Table S4) to result in the following set of dimensionless equations:

$$\begin{aligned}
\epsilon_{GRK} \cdot \frac{\partial GRK}{\partial t} &= \frac{\partial^2 GRK}{\partial x^2} - \varphi_{GRK}^2 \cdot (GRK - h^+(x_{GRK} - x)) \\
\epsilon_{DPP} \cdot \frac{\partial DPP}{\partial t} &= \frac{\partial^2 DPP}{\partial x^2} - \varphi_{DPP}^2 \cdot DPP \cdot TKV \\
\epsilon_{PNT} \cdot \frac{\partial PNT}{\partial t} &= h^+(S_{EGFR}, \theta_{S_{EGFR}, PNT}) - PNT \\
\frac{\partial br}{\partial t} &= (h^+(S_{EGFR}, \theta_{S_{EGFR}, br}) + f_{br, g.i.}) \cdot h^-(PNT, \theta_{PNT, br}) \cdot \\
&\quad f_{rep}(S_{DPP}, BRK, \theta_{S_{DPP}, br}, \theta_{BRK, br}) - br \\
\epsilon_{BR} \cdot \frac{\partial BR}{\partial t} &= br - BR \\
\epsilon_{TKV} \cdot \frac{\partial TKV}{\partial t} &= h^+(Br, \theta_{BR, TKV}) - TKV - \kappa_{d, DPP} \cdot DPP \cdot TKV \\
\epsilon_{BRK} \cdot \frac{\partial BRK}{\partial t} &= h^+(S_{EGFR}, \theta_{S_{EGFR}, BRK}) \cdot h^-(S_{DPP}, \theta_{S_{DPP}, BRK}) - BRK \\
S_{EGFR} &= \alpha'_{EGFR} \cdot GRK \\
S_{DPP} &= \alpha'_{DPP} \cdot DPP \cdot TKV
\end{aligned} \tag{S13}$$

with the following boundary conditions:

$$\left. \frac{\partial GRK}{\partial x} \right|_{x=0} = \left. \frac{\partial GRK}{\partial x} \right|_{x=1} = 0, \quad \left. \frac{\partial DPP}{\partial x} \right|_{x=0} = 1, \quad \left. \frac{\partial DPP}{\partial x} \right|_{x=1} = 0 \tag{S14}$$

and the following initial conditions (see Tables S2 and S5 for the description and values of initial conditions used):

$$\begin{aligned}
GRK_0 &= DPP_0 = PNT_0 = 0 \\
br_0 &= \alpha_{br} \cdot k_{d, br} / \gamma_{br} \\
BR_0 &= \gamma_{BR} \cdot br_0 \\
TKV_0 &= \alpha_{TKV} \cdot k_{d, TKV} / \gamma_{TKV} \\
BRK_0 &= \alpha_{BRK} \cdot k_{d, BRK} / \gamma_{BRK}
\end{aligned} \tag{S15}$$

## Selection of model parameters

### *Threshold concentrations for the switch-like gene activation and repression*

The values of various parameters such as the activation/repression thresholds are unknown (a list of all model's parameters can be found in Table S2). However, since our model is scaled so that each concentration value ranges from zero to one, determining threshold values becomes more manageable. Although the absolute values of these

threshold concentrations are unknown, their relative values with respect to each other can be inferred from *in situ* hybridization and immunohistochemistry data.

For example, since *br* pattern extends further from the dorsal-anterior GRK source than the *pnt* pattern, we can conclude that expression of *pnt* requires a higher EGFR signaling level than that of *br* ( $\theta_{GRK,PNT} > \theta_{GRK,br}$ ). Additionally,  $\theta_{DPP,br}$  has to be set low enough so that at stage 11, P-MAD is capable to completely abolish the dorsolateral patches of *br*, requiring that even the low level of P-MAD in the posterior side of the patch is also capable of *br* repression. Another example is the value of  $\theta_{BR,TKV}$  that has to be set high enough so that the basal concentration of BR, originating from the GRK independent induction of *br*, is too low to induce TKV expression. Since we are only concerned with the qualitative features of the system, as long as these requirements for the ranges of parameter values are met, the specific values of parameters themselves are irrelevant.

### *Reaction-diffusion parameters*

Parameters such as the diffusivities of the ligands, and constants associated with ligand-receptor binding are also not known and *in-vivo* measurements of these values are currently not feasible due to technical limitations. However, by scaling our equations, we no longer need to know the individual values of the parameters (1). As shown in Equations S13, the parameters are lumped into dimensionless numbers as the following:

$$\begin{aligned} \varepsilon_{GRK} \cdot \frac{\partial GRK}{\partial t} &= \frac{\partial^2 GRK}{\partial x^2} - \phi_{GRK}^2 \cdot (GRK - h^+(x_{GRK} - x)) \\ \varepsilon_{DPP} \cdot \frac{\partial DPP}{\partial t} &= \frac{\partial^2 DPP}{\partial x^2} - \phi_{DPP}^2 \cdot DPP \cdot TKV \end{aligned} \quad [S16]$$

Note that now, for each equation, all relevant parameter values are grouped into only two dimensionless parameters  $\varepsilon$  and  $\phi$ . The values for  $\phi_{GRK}$  and  $\phi_{DPP}$  have been estimated using both experimental and computational methods (1, 2) and  $\varepsilon_{GRK}$  and  $\varepsilon_{DPP}$  need to be smaller than  $\varepsilon_{TKV}$  to ensure that the ligand gradients adjust quickly to changes in receptor distribution, as previously assumed (1, 2)

### *Time constants*

The absolute stability of each component *in-vivo* is mostly not known. From the *in situ* hybridization and immunohistochemistry data, we can infer that BR protein is more stable than its mRNA since the protein is still detected at stage 11 but not the mRNA. However, due to the lack of antibodies to detect the proteins of other network components, we have no information of the relative stabilities of each protein with respect to its mRNA. In addition, literature data on the relative life times among network components are lacking. Thus, for simplicity, we assumed that all the network components have similar stability and degrade at the same rate, with the exception of BR protein which has a higher stability and thus degrade at a slower rate. For this model,  $k_{d,BR} = 0.1 \cdot k_{d,br}$ . A value of  $k_{d,BR}$  that is significantly larger will result in BR being degraded before stage 11 of oogenesis, inconsistent with experimental findings.

In this model, we have assumed that the inducing or repressing effect of a regulatory arrow is instantaneous, which is clearly an approximation. This assumption causes several discrepancies between model predictions and experiments. For example, in the wild type, even though the two-patched expression of *tkv* is found in stage 10B, the two ‘eyebrow’ like domains of P-MAD expression is not seen until stage 11. This lag, which can be caused by the time lag caused by the protein translation, for example, is not incorporated in the model (the presence of this translational time lag can be confirmed once staining the TKV protein *in vivo* is feasible). Consequently, the model predicts that the eyebrow-shaped P-MAD expression appears as soon as the two patches of *tkv* appear. Similarly, since the repression of P-MAD on *brk* is assumed instantaneous, *brk* expression is complementary to that of P-MAD at all times, leading to discrepancies in *brk* expression in stages 10A-B of oogenesis as well.

### Analyses of mutant backgrounds

Here we provide more detailed analyses of modeling mutant backgrounds. In the first background, the Ras hypomorph mutant,  $\alpha_{EGFR} < 1$ . We found that values of  $\alpha_{EGFR}$  between 0.5 to 0.6 recapitulate the experimental results of BR and P-MAD in this background (Figs 3A, S1A-B). The overall reduction of EGFR signaling leads to the loss of *pnt* expression and the anterior shift of *br*, BR, *tkv* and P-MAD, and *brk* patterns, in agreement with the experimental results (Table S3).

The second example of a mutant background is provided by analysis of the effects of an anterior *pnt* clone (Fig 3C). Similar to the modeling of other clones, this *pnt* clone is generated by setting the initial value of PNT to be zero and setting the rate of change of PNT concentration to be zero locally ( $\partial[PNT]/\partial t = 0$  where the clone is located at all times). Note that since the model is formulated only for stages 9-11, clones generated in the model only appear after stage 9 and their effects can only be seen in subsequent stages of oogenesis. This is in contrast to the experimentally generated clones that normally appear rather early in oogenesis. In this background, the loss of anterior *pnt* leads to ectopic expression of *br*, BR, *tkv*, and P-MAD (Fig S1C-D).

### Modeling the DPP signaling positive feedback loop

As mentioned in the text, since the mechanism of the positive feedback loop is unknown, there are multiple ways to model it. Here we show two different methods of modeling the feedback loop and show that both lead to the same results (Fig S2). The first method involves changing the production rate of *tkv* as a function of P-MAD level. In this case, when  $P-MAD < 0.001$  (a very small concentration),  $\gamma_{TKV, PMAD < 0.001} = 0.1 * \gamma_{TKV, PMAD \geq 0.001}$ . This method predicts a reduction of *tkv* expression in CY2-DAD background, as experimentally observed (Fig S2). The second method involves changing the degradation rate of *tkv* as a function of P-MAD level. In this case, when  $P-MAD < 0.001$  (a very small concentration),  $k_{d, TKV, PMAD < 0.001} = 10 * k_{d, TKV, PMAD \geq 0.001}$ . This method also results in a reduction of *tkv* expression in DAD overexpression background (Fig S2).

## **Numerical solution**

The reaction-diffusion equations in the model are discretized by finite differences (with 41 uniformly spaced nodes). The resulting system of coupled ordinary differential equations is solved numerically in ode15s initial value problem solver in MATLAB (The MathWorks, Inc., Natick, MA). The computation was performed for a time window that is equal to the degradation time scale of BR. The results were then plotted at multiple time points and for presentation purposes, four time points were selected to represent stages 9 through 11. The overall time window of simulations  $T=450$ , is divided into 4 intervals with the relative widths that correspond to the four relevant stages of oogenesis: stages 9 and 10A are 6 hours each, stage 10B is 4 hours, and stage 11 is 30 minutes (3).



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