Suplemental Information: BRAVO and WOX5 control root survival from the stem cell niche

Nadja Bosch,[1] Isabel Betegón-Putze,[1] Josep Mercadal,[2,3] Ainoa Planas-Riverola,[1] Josep Vilarrasa-Blasi,[1,4] David Frigola,[2] Rebecca Corinna-Burkart,[5] Cristina Martínez,[6] Yvonne Stahl,[5] Salomé Prat,[6] Marta Ibañes,[2,3] and Ana I. Caño-Delgado[1]

[1] *Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB (Cerdanyola del Vallès), 08193 Barcelona, Spain.*

[2] *Departament de Física de la Matèria Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain.*

[3] *Universitat de Barcelona Institute of Complex Systems, 08028 Barcelona, Spain.*

[4] *Present address: Carnegie Institution for Science, Department of Plant Biology, 260 Panama St. Stanford, CA 94305, USA*

[5] *Institute for Developmental Genetics, Heinrich Heine University, Universitätsstraße 1, Düsseldorf 40225, Germany.*

[6] *Department of Plant Molecular Genetics, Centro Nacional de Biotecnología (CNB), Darwin 3, Madrid E-28049, Spain.*

MATHEMATICAL MODEL

We use a mathematical model to analyze the interplay between two proteins, BRAVO and WOX5 (M and W, respectively), and the effect of this interplay on steady state promoter activities and protein concentrations. With a series of hypotheses, we connect the steady states with the experimental data of the activity of the promoters of both genes.

The model consists on transcriptional interactions as well as on the formation of complexes between proteins, in the following way: BRAVO can bind to its own promoter and to the promoter of WOX5, increasing their activity; WOX5, for its part, can bind to the BRAVO promoter, also acting as an activator. Binding sites are assumed to act independently from the others, so that there are binding sites specific to BRAVO and binding sites specific to WOX5. Finally, the two proteins can form a complex and are assumed to remain bound, never detaching back. The complex does not bind to the promoter of BRAVO or WOX5. Hence, complex formation sequesters the free proteins, which are assumed to be the active ones controlling transcription. With these assumptions, the model reads:

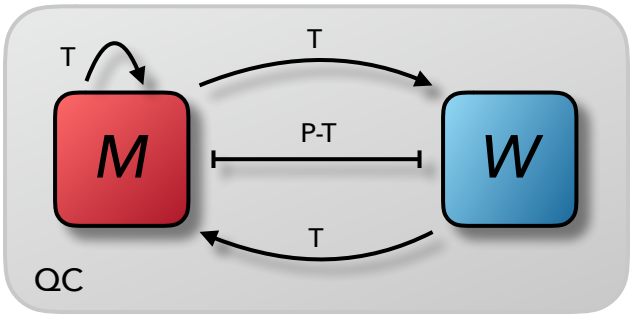
$$\frac{dM}{dt} = \bar{\alpha}\left(\frac{1+\varepsilon_{MM}K_{MM}M}{1+K_{MM}M}\right)\left(\frac{1+\varepsilon_{WM}K_{WM}W}{1+K_{WM}W}\right) - \lambda_{MW}MW - d_M M \tag{1}$$

$$\frac{dW}{dt} = \bar{\gamma}\left(\frac{1+\varepsilon_{MW}K_{MW}M}{1+K_{MW}M}\right) - \lambda_{MW}MW - d_W W \tag{2}$$

A list of the model's parameters is shown in the table of Figure 1. The first two terms in paranthesis of Eq. (1) on the right hand side is proportional to the promoter activity of BRAVO, which depend upon the binding of WOX5 and BRAVO proteins, acting as activators ($\varepsilon_{MM} > 1$, $\varepsilon_{WM} > 1$). The next term represents the loss of BRAVO proteins due to the formation of complexes with WOX5 (measured by λ_{MW}). The last term accounts for linear degradation of the protein, measured by d_M. In Eq. (2) on the right hand side, the first term is proportional to the WOX5 promoter activity, with BRAVO acting as an activator ($\varepsilon_{MW} > 1$). Finally, the remaining terms, as before, represent complex formation and degradation. In writing the previous equations we have assumed that mRNA dynamics are very fast compared to the dynamics of transcription, so that the effect of translation is modelled without the mRNA as an intermediate. Therefore, the parameters $\bar{\alpha}$ and $\bar{\gamma}$, interpreted here simply as protein production, can be rethought as the translation rates with mRNA in the steady state. Transcription, for its part, is modelled through Michaelis-Menten kinetics, where binding and unbinding of proteins remain in chemical equilibrium, with $1/K_{XY}$ representing the dissociation constant, i.e. the concentration of free X at which half of the total amount of Y is bound with protein X. The complexes are only formed pair-wise and with different proteins (so no complex of the form M^2 or W^2 is allowed), and degradation of free proteins is assumed to be linear.

We will compare the steady states of the previous equations in WT conditions and in *bravo* and *wox5* loss of function

mutants to see how they are able to match experimental data. In these mutants, the corresponding proteins are not functional, so that in the equations every term in which the protein plays a role can be set to zero. The analysis of



PARAMETER	DESCRIPTION
$\bar{\alpha}$	Basal production rate of BRAVO proteins
$\bar{\gamma}$	Basal production rate of WOX5 proteins
K_{MM}	Binding/unbinding ratio of BRAVO to pBRAVO
K_{MW}	Binding/unbinding ratio of BRAVO to pWOX5
K_{WM}	Binding/unbinding ratio of WOX5 to pBRAVO
λ_{MW}	Rate of WOX5/BRAVO complex formation
ε_{MM}	Fold-change increase of pBRAVO by BRAVO
ε_{WM}	Fold-change increase of pWOX5 by BRAVO
ε_{MW}	Fold-change increase of pBRAVO by WOX5
d_X	Degradation rate of protein X

Figure 1: (Left) Diagram representing the WOX5/BRAVO system. Normal arrows indicate activation, while arrows with blunt ends indicate inhibition. The labels T and P-T denote transcriptional and post-transcriptional interactions, respectively. (Right) Parameters of the full mathematical model and their description.

the model becomes easier if we write it in non-dimensional form. Rescaling time $\tau = d_M t$ and changing variables to $\hat{M} = A_1 M$, $\hat{W} = A_2 W$, we can write

$$\frac{d\hat{M}}{d\tau} = \frac{A_1\bar{\alpha}}{d_M}\left(\frac{1+\varepsilon_{MM}\frac{K_{MM}}{A_1}\hat{M}}{1+\frac{K_{MM}}{A_1}\hat{M}}\right)\left(\frac{1+\varepsilon_{WM}\frac{K_{WM}}{A_2}\hat{W}}{1+\frac{K_{WM}}{A_2}\hat{W}}\right) - \frac{\lambda_{MW}}{A_2 d_M}\hat{M}\hat{W} - \hat{M} \tag{3}$$

$$\frac{d\hat{W}}{d\tau} = \frac{A_2\bar{\gamma}}{d_M}\left(\frac{1+\varepsilon_{MW}\frac{K_{MW}}{A_1}\hat{M}}{1+\frac{K_{MW}}{A_1}\hat{M}}\right) - \frac{\lambda_{MW}}{A_1 d_M}\hat{M}\hat{W} - \frac{d_W}{d_M}\hat{W} \tag{4}$$

Then the system can be rewritten in the following non-dimensional form:

$$\frac{dM}{dt} = \alpha\left(\frac{1+\varepsilon_{MM}M}{1+M}\right)\left(\frac{1+\varepsilon_{WM}W}{1+W}\right) - \lambda MW - M \tag{5}$$

$$\delta^{-1}\frac{dW}{dt} = \gamma\left(\frac{1+\varepsilon_{MW}qM}{1+qM}\right) - \epsilon\lambda WM - W \tag{6}$$

Where we have set $A_1 = K_{MM}$, and $A_2 = K_{WM}$, and defined $\alpha = \dfrac{K_{MM}\bar{\alpha}}{d_M}$, $\gamma = \dfrac{K_{WM}\bar{\gamma}}{d_W}$, $\lambda = \dfrac{\lambda_{MW}}{K_{WM}d_M}$, $\epsilon = \dfrac{K_{WM}d_M}{K_{MM}d_W}$, $q = \dfrac{K_{MW}}{K_{MM}}$. The ratio $\delta = d_W/d_M$ sets the time-scale of W relative to M. With the previous nondimensionalization, we reduced the number of parameters by two. The first terms on the right hand side of equations (5) and (6) are interpreted as *promoter activities* of BRAVO and WOX5, respectively, and are defined as the following quantities:

$$F_M = \alpha\left(\frac{1+\varepsilon_{MM}M}{1+M}\right)\left(\frac{1+\varepsilon_{WM}W}{1+W}\right), \quad F_W = \gamma\left(\frac{1+\varepsilon_{MW}qM}{1+qM}\right) \tag{7}$$

These functions will allow us make a direct connection between theory and experiments by computing their fold-changes in mutant genotypes with respect to wild-type genotypes. We are interested in the steady states of the system (5)(6),

α	γ	λ	ϵ	q	δ
$\dfrac{K_{MM}\bar{\alpha}}{d_M}$	$\dfrac{K_{WM}\bar{\gamma}}{d_W}$	$\dfrac{\lambda_{MW}}{K_{WM}d_M}$	$\dfrac{K_{WM}d_M}{K_{MM}d_W}$	$\dfrac{K_{MW}}{K_{MM}}$	$\dfrac{d_W}{d_M}$

Table I: Nondimensional parameters and their relations with the original model's parameters.

which are expected to correspond to the experimental observations.

Because both proteins activate each other's transcription, an immediate consequence of the model is that when a protein (say M) is non-functional (e.g. in mutant genotypes), the production of the other (W) decreases, as there is no transcriptional activation. However, in the mutants there is no protein sequestration by the formation of the BRAVO-WOX5 complex. These opposite effects in mutants can be understood by looking at the nullclines of the system (Figure 2). We see that complex formation can induce opposite effects on proteins if the rate is large enough. If no complex is formed, $\lambda = 0$, then nullclines are always increasing functions of its corresponding variable, meaning that proteins will always be activating. However, for $\lambda > 0$, there are two regimes, one in which the steady state value of proteins increases upon an increment of proteins, and one in which an increase in proteins produces a decrease in the steady state value of proteins. This happens because complex formation always increases as proteins increase, while promoter activities reach a maximum. Therefore, when this maximum value of promoter activity is reached−all binding sites are occupied by proteins−the effect of increasing proteins will only induce more complex formation, thereby decreasing the steady state value.

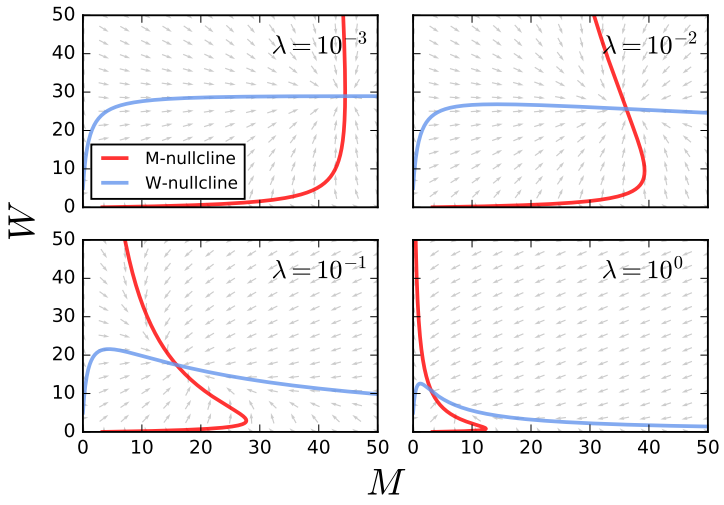


Figure 2: Nullclines and vector field for the BRAVO/WOX5 model with parameters $\alpha = 1$, $\gamma = 5$, $\varepsilon_{MM} = 4$, $\varepsilon_{WM} = 12$, $\varepsilon_{MW} = 6$ and $\epsilon = 0.4$ for different values of λ. Notice how as λ increases, the protein concentration at which the two proteins begin to inhibit each other becomes smaller. This parameter regime is not the one used in the model, but only shown to illustrate the effect of complex formation on the dynamics of the system.

MUTANTS

In the mutants, since proteins are non-functional we can set them to zero, simplifying the system significantly. The equations of the system (5)(6) are transformed to:

$$\frac{dW}{dt} = \gamma - W, \qquad \text{in the } \textit{bravo} \text{ mutant} \tag{8}$$

$$\frac{dM}{dt} = \alpha\left(\frac{1 + \varepsilon_{MM} M}{1 + M}\right) - M, \qquad \text{in the } \textit{wox5} \text{ mutant} \tag{9}$$

Eventhough in the mutants the proteins are set to zero due to their non-functionality in controlling transcription, promoter activities are not. In particular, they are:

$$F_M^{bm} = \alpha\left(\frac{1 + \varepsilon_{WM} W_s^{bm}}{1 + W_s^{bm}}\right), \quad F_W^{bm} = \gamma, \qquad \text{in the } \textit{bravo} \text{ mutant} \tag{10}$$

$$F_M^{wm} = \alpha\left(\frac{1 + \varepsilon_{MM} M_s^{wm}}{1 + M_s^{wm}}\right), \quad F_W^{wm} = \gamma\left(\frac{1 + \varepsilon_{MW} q M_s^{wm}}{1 + q M_s^{wm}}\right), \qquad \text{in the } \textit{wox5} \text{ mutant} \tag{11}$$

Where M_s^{wm} and W_s^{bm} denote, respectively, the steady state concentration of BRAVO in the *wox5* mutant and the steady state concentration WOX5 in the *bravo* mutant, whose expressions are:

$$M_s^{wm} = \frac{\alpha\varepsilon_{MM} - 1 + \sqrt{(\alpha\varepsilon_{MM} - 1)^2 + 4\alpha}}{2}, \quad W_s^{bm} = \gamma \tag{12}$$

Comparing promoter activities in mutants with wild-type genotypes, we can make a connection with experimental data and constrain the parameters of the model to fit such data.

SELECTION OF PARAMETER VALUES CONSTRAINED BY EXPERIMENTAL DATA

The connection between theory and experiments can be done through the computation of the fold-changes between promoter activities in the wild-type and mutants. Here we use approximations and numerical simulations to constrain the parameters of the model to fit the data on promoter activities. In the *bravo* mutant, for biologically reasonable values of α we can assume that $\alpha\varepsilon_{MM} \gg 1$ and therefore we can write:

$$M_s^{wm} \simeq \alpha\varepsilon_{MM}, \quad W_s^{bm} = \gamma \tag{13}$$

Therefore the promoter activities in the *bravo* mutant will be:

$$F_M^{bm} = \alpha\frac{1 + \varepsilon_{WM}\gamma}{1 + \gamma}, \quad F_W^{bm} = \gamma \tag{14}$$

In the *wox5* mutant, we will have:

$$F_M^{wm} = \alpha\varepsilon_{MM}, \quad F_W^{wm} = \gamma\frac{1 + \varepsilon_{MW}q\alpha\varepsilon_{MM}}{1 + q\alpha\varepsilon_{MM}} \tag{15}$$

The quantities of interest here will not be promoter activities, but the *fold-changes* of such activities between mutants and WT genotypes, as these are the meaningful quantities which will allow the connection between theory and experiments. On the one hand, since the model is adimensional, the values of promoter activities are arbitrary, while fold-changes are not, because they do not depend on the scaling we performed on the variables and parameters. On the other hand, the experimental measures of promoter activity are cast into pixel intensity, and therefore are arbitrary as well. What is not arbitrary, however, is the fold-change of such intensities with respect to a given normalized value, taken to be the WT. With this, we define the fold-change between mutant and WT promoter activities as:

$$\sigma_X \equiv \frac{F_X^{mutant}}{F_X^{wt}} \tag{16}$$

That is $\sigma_X > 1$ when the mutant genotype has more promoter activity than the WT and $\sigma_X < 1$ otherwise. Written in full detail:

$$\sigma_M \equiv \frac{F_M^{bm}}{F_M^{wt}} = \frac{\left(\frac{1 + \varepsilon_{WM}\gamma}{1 + \gamma}\right)}{\left(\frac{1 + \varepsilon_{MM}M_s^{wt}}{1 + M_s^{wt}}\right)\left(\frac{1 + \varepsilon_{WM}W_s^{wt}}{1 + W_s^{wt}}\right)}, \qquad \sigma_W \equiv \frac{F_W^{wm}}{F_W^{wt}} = \frac{\left(\frac{1 + \varepsilon_{MW}q\alpha\varepsilon_{MM}}{1 + q\alpha\varepsilon_{MM}}\right)}{\left(\frac{1 + \varepsilon_{MW}qM_s^{wt}}{1 + qM_s^{wt}}\right)} \tag{17}$$

$$\sigma_M^\dagger \equiv \frac{F_M^{wm}}{F_M^{wt}} = \frac{\varepsilon_{MM}}{\left(\frac{1 + \varepsilon_{MM}M_s^{wt}}{1 + M_s^{wt}}\right)\left(\frac{1 + \varepsilon_{WM}W_s^{wt}}{1 + W_s^{wt}}\right)}, \qquad \sigma_W^\dagger \equiv \frac{F_W^{bm}}{F_W^{wt}} = \frac{1}{\left(\frac{1 + \varepsilon_{MW}qM_s^{wt}}{1 + qM_s^{wt}}\right)} \tag{18}$$

This quantity will be of interest for both mutants and promoters, since these are the outputs of the experiments (Table II, Left). Computing the steady states of the WT numerically, we can find for different values of (M_s^{wt}, W_s^{wt}) which are the corresponding σ. If we impose the conditions given by the experiments, we can find restrictions on the parameters. On the one hand, it is easy to show that the following identities are true:

$$\frac{\sigma_W}{\sigma_W^\dagger} = \frac{1 + \varepsilon_{MW}q\alpha\varepsilon_{MM}}{1 + q\alpha\varepsilon_{MM}}, \quad \frac{\sigma_M}{\sigma_M^\dagger} = \frac{1}{\varepsilon_{MM}}\frac{1 + \varepsilon_{WM}\gamma}{1 + \gamma} \tag{19}$$

From experimental data (Figure 1 of the main text) we see that these ratios are:

$$\frac{\sigma_W}{\sigma_W^\dagger} \simeq 1.88, \qquad \frac{\sigma_M}{\sigma_M^\dagger} \simeq 14.29 \tag{20}$$

Therefore, fixing $\varepsilon_{MM} = 4$ (a value reported in [1]) and assuming $q\alpha, \gamma \gg 1$ (in particular, we set $q = 1$, $\alpha = 10$, $\gamma = 50$), we can easily show that the remaining parameters must be:

$$\varepsilon_{MW} \simeq 1.9, \quad \varepsilon_{WM} \simeq 57 \tag{21}$$

The assumptions $q\alpha, \gamma \gg 1$, which eliminate the dependence of α, q and γ in the ratios $\sigma/\sigma^\dagger$, are not arbitrary: in the nondimensional units we work with, the basal production rate of proteins are $\bar{\alpha} = \frac{d_M \alpha}{K_{MM}}$ and $\bar{\gamma} = \frac{d_W \gamma}{K_{WM}}$. Typical values for these parameters are $K_{MM} = K_{WM} = 100$ nM^{-1}, $d_M = d_W = 1$ h^{-1} [4], and therefore with values of $\alpha = 10$ and $\gamma = 50$ one obtains $\bar{\alpha} \sim 10^{-1}$ nM·h^{-1} and $\bar{\gamma} \sim 0.5$ nM·h^{-1}, which are of the order of typical values of protein production $10^{-1} - 10^3$ nM·h^{-1} [3]. Thus in this parameter regime the approximations $q\alpha, \gamma \gg 1$ are justified. In order to constrain the remaining parameters λ and ϵ, we rely on numerical simulations. First we numerically compute the fixed points (M_s^{wt}, W_s^{wt}) with the parameters found with the previous analysis and for different values of λ and ϵ. Then we substitute these values into the expression of promoter activities F_M^{wt} and F_W^{wt} and compute the ratios σ. These ratios are plotted in figure (Figure 3).

EFC	Value
P_M^{bm}/P_M^{wt}	2.00 ± 0.87
P_M^{wm}/P_M^{wt}	0.14 ± 0.09
P_W^{wm}/P_W^{wt}	1.35 ± 0.43
P_W^{bm}/P_W^{wt}	0.72 ± 0.23

MFC	Definition	Value
σ_M	F_M^{bm}/F_M^{wt}	2.02
$\sigma_M^\dagger$	F_M^{wm}/F_M^{wt}	0.14
σ_W	F_W^{wm}/F_W^{wt}	1.36
$\sigma_W^\dagger$	F_W^{bm}/F_W^{wt}	0.72

PFC	Value
M_s^{bm}/M_s^{wt}	0
M_s^{wm}/M_s^{wt}	53
W_s^{wm}/W_s^{wt}	0
W_s^{bm}/W_s^{wt}	208

Table II: (Left) Experimental fold-changes (EFC) in the promoter activities of mutants with respect to wild-type genotypes with their associated errors. (Center) Results of the same fold-changes computed with the model (modelling fold-changes, MFC). (Right) The corresponding protein fold-changes (PFC) in the steady-state. The nondimensional parameters used in order to obtain such results are $\alpha = 10$, $\gamma = 50$, $\varepsilon_{MM} = 4$, $\varepsilon_{WM} = 57$, $\varepsilon_{MW} = 1.9$, $q = 1$, $\lambda = 1500$, $\epsilon = 0.25$.

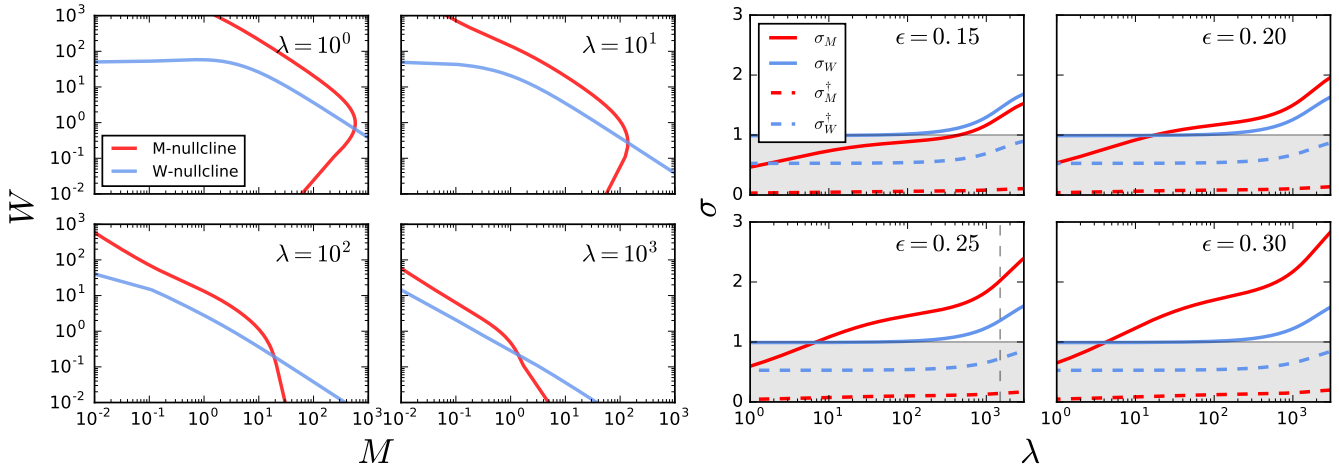


Figure 3: (Left) Nullclines of the reduced system (on a log-log scale) with parameters $\alpha = 10$, $\gamma = 50$, $\varepsilon_{MM} = 4$, $\varepsilon_{WM} = 57$ and $\varepsilon_{MW} = 1.9$, $\lambda = 10^0 - 10^3$ and $\epsilon = 0.25$. (Right) Numerical simulations with the same parameters (except λ and ϵ, which vary) showing how the ratios σ change as λ and ϵ vary. Experimentally observed results contrains these values to be $\lambda \sim 1500$ and $\epsilon \sim 0.25$, displayed in the figure with a vertical, dashed gray line.

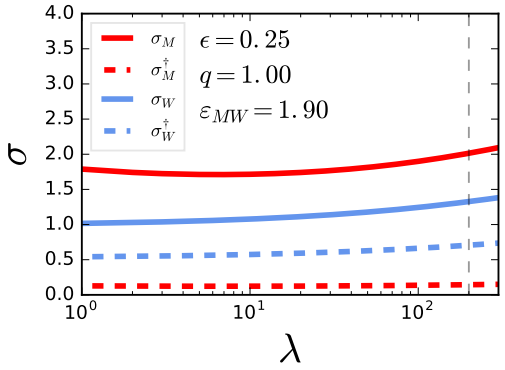
We see that in order to explain the experimental results with this model, the complex formation rate must be large, namely of the order of $\lambda \sim 1500$ and that ϵ must also be finely tuned. This is because the autoactivation of BRAVO makes more difficult for the promoter to increase in the *bravo* mutant, since the autoactivation term decreases in this mutant.

Thus, to compensate this fact, the levels of free WOX5 protein in the *bravo* mutant must be very high compared to the WT, a situation only attainable if in the WT strong complex formation prevents free proteins to be in high concentrations. In the dimensional model, the complex formation rate is given by $\lambda_{MW} = \lambda K_{WM} d_M$ and thus for $K_{WM} = 100$ nM^{-1} and $d_M = 1$ h^{-1} [4] we obtain $\lambda_{MW} = 75000$ nM^{-1}·h^{-1}, which is very large compared to the typical rates of protein complex formation, $10^{-1} - 10^{3}$ nM^{-1}·h^{-1} [3][8]. Moreover, since in our model we have not considered the unbinding of the protein complex into its constituent proteins, which accounts for an extra term in the production of M and W, the value of λ_{MW} must be a lower bound (i.e. the limit in which the unbinding of the complex is zero) of the actual value we would obtain if we considered such a process. A possible way to reduce this value would be considering the binding sites of the promoters to be non-independent, so both proteins can share binding sites. The equations of the model would be:

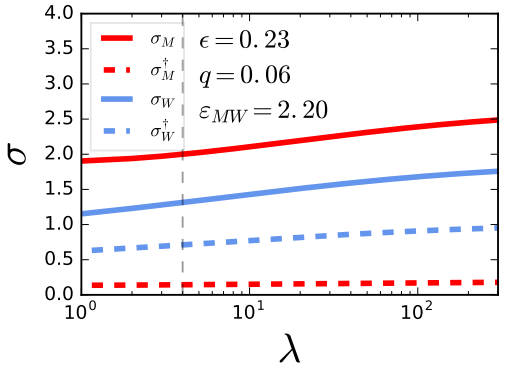
$$\frac{dM}{dt} = \alpha \frac{1 + \varepsilon_{MM} M + \varepsilon_{WM} W}{1 + M + W} - \lambda MW - M \tag{22}$$

$$\delta^{-1} \frac{dW}{dt} = \gamma \frac{1 + \varepsilon_{MW} q M}{1 + qM} - \epsilon \lambda W M - W \tag{23}$$

Now transcriptional regulation by several proteins is not additive as in the independent binding case, since the same binding site can be occupied by both proteins. However, in mutant genotypes there is no difference between this model and the original, and therefore Eqs. (14) and (15) still apply, thereby obtaining the same values for ε_{WM} and ε_{MW}, but different values for λ and ϵ (Figure 4 Left). In particular, for the same parameter set as in Table II, we obtain



MFC	Definition	Value	PFC	Value
σ_M	F_M^{bm}/F_M^{wt}	2.01	M_s^{bm}/M_s^{wt}	0
$\sigma_M^\dagger$	F_M^{wm}/F_M^{wt}	0.14	M_s^{wm}/M_s^{wt}	45.2
σ_W	F_W^{wm}/F_W^{wt}	1.32	W_s^{wm}/W_s^{wt}	0
$\sigma_W^\dagger$	F_W^{bm}/F_W^{wt}	0.71	W_s^{bm}/W_s^{wt}	30.7



MFC	Definition	Value	PFC	Value
σ_M	F_M^{bm}/F_M^{wt}	2.00	M_s^{bm}/M_s^{wt}	0
$\sigma_M^\dagger$	F_M^{wm}/F_M^{wt}	0.14	M_s^{wm}/M_s^{wt}	4.6
σ_W	F_W^{wm}/F_W^{wt}	1.31	W_s^{wm}/W_s^{wt}	0
$\sigma_W^\dagger$	F_W^{bm}/F_W^{wt}	0.71	W_s^{bm}/W_s^{wt}	6.3

Figure 4: Fold-changes computed numerically in the independent binding case for different values of ϵ, q and ε_{MW} as the parameter λ changes. The vertical, dashed gray lines denote the experimentally observed values. (Top left) If promoter binding sites are not independent, with the same parameters as the original model, the value of λ can be reduced up to $\lambda = 200$. (Top right) Changing other parameters such as q and ε_{MW} makes λ to decrease further, up to $\lambda = 4$. (Bottom) Corresponding promoter and protein fold-changes for the two cases.

$\lambda \simeq 200$ and $\epsilon \simeq 0.25$. The decrease of λ with respect to the original model reflects the fact that competence between binding sites induces an effective autorepression of BRAVO, consequence of the disparity between ε_{WM} and ε_{MM}, namely $\varepsilon_{WM} \gg \varepsilon_{MM}$. This can be seen in Figure 5 (Right), where the promoter activity of BRAVO is plotted (blue surface) in the (M, W, P_M) space: for a given value of W, the promoter activity of BRAVO seems to decrease as M increases. The exact value of W at which BRAVO switches from being an autoactivator to an autorepressor can be computed by taking the partial derivative of P_M with respect to M, obtaining the following threshold value:

$$W_t = \frac{1 - \varepsilon_{MM}}{\varepsilon_{MM} - \varepsilon_{WM}} \simeq 0.057 \tag{24}$$

Thus, for $W < W_t$ BRAVO is an autoactivator and for $W > W_t$ an autorepressor. For $W = W_t$ BRAVO has no effect on P_M. Since this threshold value is very small compared to the typical values we obtain for W with the standard set of

parameters, BRAVO almost always behaves as an autorepressor.

Recall that in the independent binding case, strong complex formation was needed to overcome the mutual transcriptional activation between BRAVO and WOX5 and the autoactivation of BRAVO, thereby obtaining the correct fold-changes. Now, since BRAVO effectively acts as an autorepressor, an increase of the BRAVO promoter activity in the *bravo* mutant will follow directly, thus relaxing the need for strong complex formation. We can further reduce the value of $\lambda = 200$ if we decrease the value of q and consequently the value of ε_{MW}, as in this case the approximation made in Eq. (21) is no longer valid. As seen in Figure 4 (Right), with $\epsilon = 0.23$, $q = 0.06$ and $\varepsilon_{MW} = 2.2$ we obtain a value of $\lambda = 4$. In terms of dimensional quantities, this corresponds to $\lambda_{MW} \simeq 200$ nM^{-1}·h^{-1}, which is in the range $10^{-1} - 10^{3}$ nM^{-1}·h^{-1} of experimentally observed rates of complex formation.

The fact that decreasing q leads to a smaller value of λ can be illustrated as follows: since $q = \frac{K_{MW}}{K_{MM}}$ measures the tendency of BRAVO proteins to bind to the WOX5 promoter with respecto to the BRAVO promoter, a small value of q reflects the fact that the amount of BRAVO needed to activate WOX5 has to be large. In turn, this high amount of BRAVO will repress the BRAVO promoter activity so much that low complex formation will be needed to obtain the desired fold-changes in promoter activity.

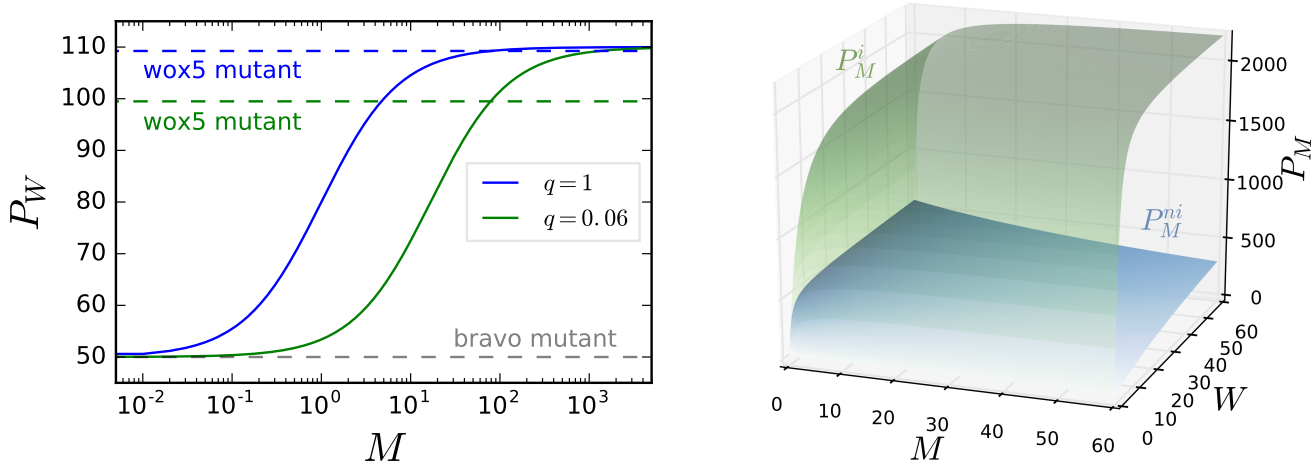


Figure 5: (Left) Promoter activity of WOX5 for two different values of $q = 1$ (blue) and $1 = 0.06$ (green) and their respective mutant promoter activities (dashed lines). (Right) Promoter activity of BRAVO when promoter binding sites are independent (P_M^i, green) and not independent (P_M^{ni}, blue). We see that in the independent binding case proteins are always activating, while in the non-independent binding case BRAVO acts effectively as a repressor, due to competence between binding sites.

One has to point out that the errors associated with experimental measures are large, and in the context of constraining the model, a small deviation from the mean value can account for a large change in the parameters. Therefore, when we say that $\lambda = 4$ we have to acknowledge the possible deviations from this value. By looking at Figure 4, we can state that these deviations can span up to two orders of magnitude.

ALTERNATIVE MODELS

The proposed model is able to reproduce experimental results and predict, accordingly, how proteins are regulated. A first observation concerns the uniqueness of such a model, namely the fact that other models or variants of the original one may, in principle, be able to reproduce experimental results as well. In this section we review some of these models and discuss whether or not they are suitable for explaining the experimentally observed data.

Mutual activation and self-repression

An alternative approach which would allow us to recover the expermental observations would be the following. Assume that BRAVO and WOX5 act as their own repressors and are activators of each other, without forming a complex. If promoter binding sites are independent, the model equations would read:

$$\frac{dM}{dt} = \alpha\left(\frac{1+\varepsilon_{MM}M}{1+M}\right)\left(\frac{1+\varepsilon_{WM}W}{1+W}\right) - M \tag{25}$$

$$\delta^{-1}\frac{dW}{dt} = \gamma\left(\frac{1+\varepsilon_{MW}qM}{1+qM}\right)\left(\frac{1+\varepsilon_{WW}pW}{1+pW}\right) - W \tag{26}$$

but now with $\varepsilon_{MM} < 1$, $\varepsilon_{WM} > 1$, $\varepsilon_{MW} > 1$ and $\varepsilon_{WW} < 1$, so that the conditions of mutual activation and autorepression are fulfilled. It is not hard to notice that such interactions lead *directly* to the experimentally observed behaviors, as long as the previous constraints for the ε hold. If the BRAVO protein M is both the activator of WOX5 and its own repressor, then in the *bravo* mutant the BRAVO promoter activity will inevitably increase with respect to the WT, due to the absence of functional BRAVO ($M = 0$ in the equations), while WOX5 promoter activity will necessarily decrease. Moreover, in this model the steady state protein concentrations have the the same value as their promoter activities, and thus protein fold-changes are the same as promoter activity fold-changes. For example, the parameter set $\alpha = 10$, $\gamma = 50$, $\varepsilon_{MM} = 0.5$, $\varepsilon_{WW} = 0.7$, $\varepsilon_{MW} = 1.4$, $\varepsilon_{WM} = 7$, $p = q = \delta = 1$, gives the desired results, as seen in Table III.

MFC	Definition	Value
σ_M	F_M^{bm}/F_M^{wt}	1.94
$\sigma_M^\dagger$	F_M^{wm}/F_M^{wt}	0.16
σ_W	F_W^{wm}/F_W^{wt}	1.37
$\sigma_W^\dagger$	F_W^{bm}/F_W^{wt}	0.72

PFC	Value
M_s^{bm}/M_s^{wt}	0
M_s^{wm}/M_s^{wt}	0.16
W_s^{wm}/W_s^{wt}	0
W_s^{bm}/W_s^{wt}	0.72

Table III: (Left) Fold-changes computed with the alternative model. (Right) The corresponding protein fold-changes (PFC) in the steady-state. The nondimensional parameters used in order to obtain such results are $\alpha = 10$, $\gamma = 50$, $\varepsilon_{MM} = 0.5$, $\varepsilon_{WW} = 0.7$, $\varepsilon_{MW} = 1.4$, $\varepsilon_{WM} = 7$, $p = q = \delta = 1$.

There are at least two reasons for discarding this model: first, complex formation has been measured experimentally, and therefore in principle we cannot ignore its effects. It could happen, however, that the rate of complex formation is so small compared to transcriptional dynamics that the net effect on promoter activities can be considered negligible. In such a case, the previous model would be a good approximation. Second, BRAVO has been reported to be an autoactivator, not an autorepressor [1], and such a change would make the model inadequate to reproduce experimental data.
It's important to stress that if complexes are formed a clear interpretation of the increased protein levels in mutants arises, namely, as a compensatory mechanism for cell survival upon damage, counteracting the loss of mutated, nonfunctional ones. In the absence of complex formation, proteins would decrease in mutants, therefore forcing us to abandon this interpretation.

The complex acting as a transcription factor

In this approach the we explore what happens if the complex acts as a transcription factor, activating or repressing the production of BRAVO and/or WOX proteins. If we assume independent binding to the promoter, the general form of the model reads:

$$\frac{dM}{dt} = \alpha\left(\frac{1+\varepsilon_{MM}M}{1+M}\right)\left(\frac{1+\varepsilon_{WM}W}{1+W}\right)\left(\frac{1+\varepsilon_{CM}rMW}{1+rMW}\right) - \lambda MW - M \tag{27}$$

$$\delta^{-1}\frac{dW}{dt} = \gamma\left(\frac{1+\varepsilon_{MW}qM}{1+qM}\right)\left(\frac{1+\varepsilon_{WW}pW}{1+pW}\right)\left(\frac{1+\varepsilon_{CW}sMW}{1+sMW}\right) - \epsilon\lambda MW - W \tag{28}$$

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