The Drosophila gap gene network is composed of two parallel toggle switches

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1. Quantitative framework – binding site occupancy models

Given concentration [*A*] of a transcriptional activator A and a binding affinity *K* of a site for A, probability to occupy this site *p* is equal to:

$$p^A([A],K)=\frac{K[A]}{1+K[A]};\quad K=e^{\frac{-\Delta G}{RT}} \tag{S1}$$

For an array of N equal sites, each with binding affinity constant K, probability that at least one site in that array will be occupied by TF (non-empty states) is equal to:

$$p^A([A],K,N)=\frac{(1+K[A])^N-1}{(1+K[A])^N} \tag{S2}$$

For an array of *N* cooperating ($C=e^{\frac{-\Delta G}{RT}}$, *C* - fold of increased binding) equal binding sites, the probability of occupancy of at least one site is equal to:

$$p^A([A],K,C,N)=\frac{(1+CK[A])^N-1}{C+(1+CK[A])^N-1} \tag{S3}$$

Within this framework, equation S3 is proportional to the probability of activation (rate of synthesis) of a gene, regulated by the transcriptional activator A. If *R* is a transcriptional repressor, then the probability of repression of the downstream gene is equal to:

$$p^R([R],K,C,N)=1-\frac{(1+CK[R])^N-1}{C+(1+CK[R])^N-1}=\frac{C}{C+(1+CK[R])^N-1} \tag{S4}$$

If gene expression is outcome of several regulatory events, all required (mode “AND”), then the synthesis rate of that gene *P* is given by the product of activation from *i* site arrays for *i* activators and repression from *j* site arrays for *j* repressors as follows:

$$P=\prod_i p_i^A\prod_j\left(1-p_j^R\right) \tag{S5}$$

If gene expression is the result of *i* activatory events, which supplement each other, but not ultimately required (mode “OR”), then the synthesis rate of the downstream gene is proportional to:

$$P=1-\prod_i\left(1-p_i^A\right) \tag{S6}$$

Eq. S1-S6 have been described elsewhere [1,2].

2. Detailed models describing positional cues for maternal and gap genes

Hunchback. Bicoid and Hunchback itself regulate expression of *hunchback*; the both regulators are required (operator AND) for Hunchback expression:

$$P^{Hb} = p^{Bcd}\, p^{Hb} \tag{S7}$$

Substitution using eq S3 for a cooperative array of activator binding sites returns:

$$P^{Hb} = \frac{\left(1 + C^{Bcd} K^{Bcd}[Bcd]\right)^{N^{Bcd}} - 1}{C^{Bcd} + \left(1 + C^{Bcd} K^{Bcd}[Bcd]\right)^{N^{Bcd}} - 1} * \frac{\left(1 + C^{Hb} K^{Hb}[Hb]\right)^{N^{Hb}} - 1}{C^{Hb} + \left(1 + C^{Hb} K^{Hb}[Hb]\right)^{N^{Hb}} - 1} \tag{S7a}$$

Notice, any other regulatory link, including Bicoid-activator will carry exactly the same Bicoid-specific parameter values. This emulates an assumption that every gene activated by Bicoid carries exactly the same array of Bicoid binding sites. However, in the case of Bicoid repression (see eq. S8 below) the Bicoid-specific parameters (*K*, *C*, *N*) were allowed to be different (but were the same in the actual models). This is true for every other transcriptional regulator (node) in the integrated model. For instance, Hunchback acting as activator or dual regulator has one sets of constants (*K*, *C*, *N*), Hunchback acting as a repressor was allowed to have different constants. (see Figure 3 in the main text, Figure S1 and eq. S9-S10 below).

Caudal is repressed by Bicoid translationally, however the same framework has been applied to this network connection, given that Bicoid directly binds sites in *caudal* 3' mRNA:

$$P^{Cad} = 1 - p^{Bcd-R} \tag{S8}$$

$$P^{Cad} = \frac{C^{Bcd-R}}{C^{Bcd} + \left(1 + C^{Bcd-R} K^{Bcd-R}[Bcd]\right)^{N^{Bcd-R}} - 1} \tag{S8a}$$

The *caudal* model was a single steady-state model, taking place of yet another maternal input to the dynamic gap gene network model.

Kruppel is activated and repressed by Hunchback (dual regulation Hb parameters):

$$P^{Kr} = p^{Hb}\left(1 - p^{Hb}\right) \tag{S9}$$

$$P^{Kr} = \frac{\left(1 + C^{Hb} K^{Hb}[Hb]\right)^{N^{Hb}} - 1}{C^{Hb} + \left(1 + C^{Hb} K^{Hb}[Hb]\right)^{N^{Hb}} - 1} * \frac{C^{Hb}}{C^{Hb} + \left(1 + C^{Hb} K^{Hb}[Hb]\right)^{N^{Hb}} - 1} \tag{S9a}$$

Knirps is activated by Bicoid and is repressed by Hunchback (Hb-R parameters):

$$P^{Kni} = p^{Bcd}\left(1 - p^{Hb-R}\right) \tag{S10}$$

$$P^{Kni} = \frac{\left(1 + C^{Bcd} K^{Bcd} [Bcd]\right)^{N^{Bcd}} - 1}{C^{Bcd} + \left(1 + C^{Bcd} K^{Bcd} [Bcd]\right)^{N^{Bcd}} - 1} * \frac{C^{Hb-R}}{C^{Hb-R} + \left(1 + C^{Hb-R} K^{Hb-R} [Hb]\right)^{N^{Hb-R}} - 1} \quad \text{(S10a)}$$

Giant. Either Bicoid or Caudal (operator OR) activate expression of *giant*:

$$P^{Gt} = 1 - \left(1 - p^{Bcd}\right)\left(1 - p^{Cad}\right) \quad \text{(S11)}$$

$$P^{Gt} = 1 - \frac{C^{Bcd}}{C^{Bcd} + \left(1 + C^{Bcd} K^{Bcd} [Bcd]\right)^{N^{Bcd}} - 1} * \frac{C^{Cad}}{C^{Cad} + \left(1 + C^{Cad} K^{Cad} [Cad]\right)^{N^{Cad}} - 1} \quad \text{(S11a)}$$

Models eq. S7, S9, S10 have been described in details in previous publications [1,2,3]. Model eq. S8 fits well the observed distribution of Bicoid and Caudal gradients; model eq. S11 has been developed in this work based on Gt expression in Bicoid and Cad mutants.

3. Model performance and solutions

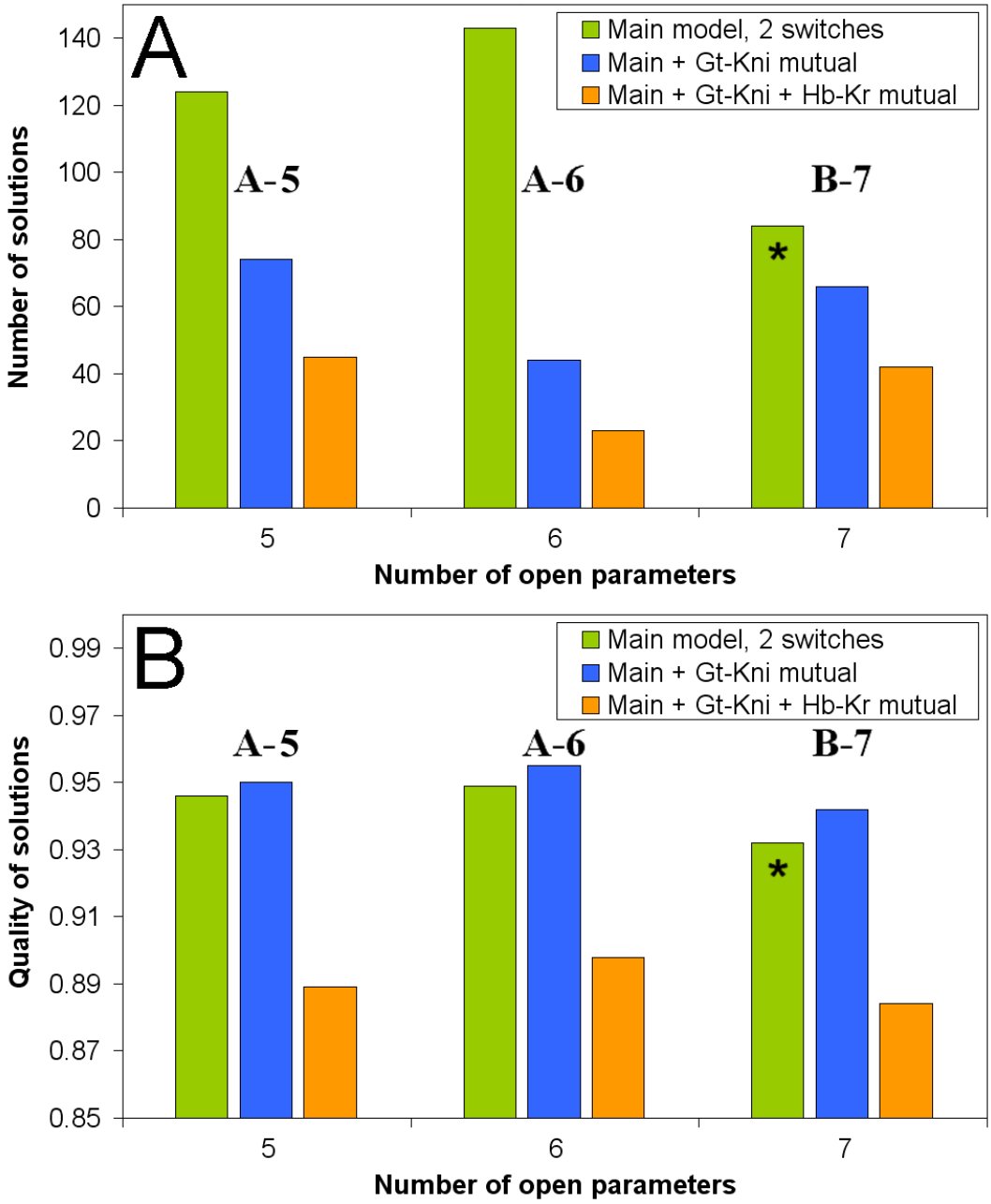

Figure S1. Performance of models with different architecture
(A) Number of solutions and (B) quality of the best solution (correlation, see Methods section) for three different variants (color coded) of the gap gene network. Star marks main model used in this study with 7 parameters open. Incorporation of Gt-Kni mutual repression (in blue) reduces the number of solutions (A), but identifies solutions with better quality (B). Incorporation of both Gt-Kni and Hb-Kr mutual repression (in orange) reduces both the number and the quality of solutions.

4. Model validation

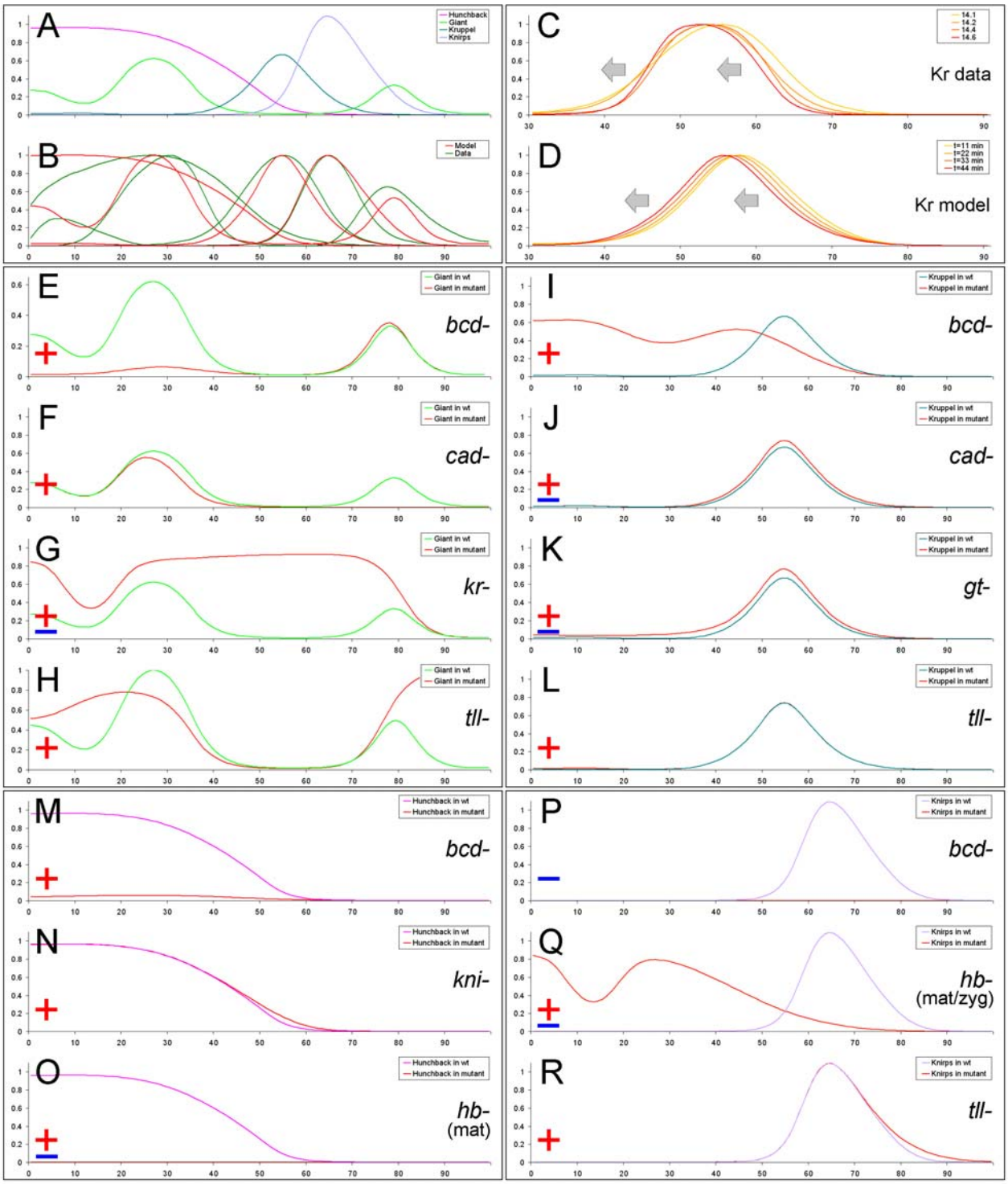

Figure S2. Simulation of mutant expression

(A) Model, (B) model-data fitting, (C-D) anterior shift of Kruppel. (E-H) Simulation of Giant expression in mutants, (I-L) Kruppel expression in mutants, (M-O) Hunchback and (P-R) Knirps expression in mutants. Most mutants, which are not shown (e.g. Hb in Kr^{-}) showed no changes from *wt* in the simulations. Many simulations are in agreement (+) with *in vivo* data [4,5,6,7,8,9,10,11,12,13,14,15,16,17,18]. In the absence of Bicoid, the anterior Giant pattern disappears; Kruppel extends to anterior (A, B); Hunchback anterior and Knirps disappear (I, J). In the absence of Caudal, the posterior Giant pattern disappears (A, C). Zygotic Hunchback disappears in the absence of maternal Hunchback (M). Knirps displays broad anterior expression pattern in the absence of both maternal and zygotic Hunchback (L). In the absence of Knirps, Hunchback anterior patter expands to posterior (K); in the absence of Kruppel, Giant has broad expression, the posterior and the anterior stripes merge (E). Removal of Tailless results in terminal expansion of Giant stripes (G); Knirps posterior stripe (N) and disruption of the central domain Kruppel stripe (H).

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