Dynamic control of positional information in the early Drosophila embryo

Supplementary Information

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This document contains a detailed description of gap gene circuit models and their parameters, as well as additional results concerning shifts of anterior and posterior gap domain boundaries based on analysis of selected gap gene circuits with or without diffusion. A summary of the results presented here can be found in Fig. 20 at the end of this document. Note that Fig. 1–4 refer to the figures of the main paper.
1 Gap Gene Circuits

1.1 Model: Basic Objects and State Variables

The basic objects of gap gene circuits are *Drosophila melanogaster* blastoderm nuclei denoted by index $i$. Anteroposterior (A–P) and dorsoventral (D–V) patterning systems are largely independent of each other in the trunk region of the blastoderm embryo. Therefore, nuclei $i$ are arranged in a one-dimensional row, where nuclei $i - 1$ and $i + 1$ are neighbours of nucleus $i$. During cycle 14A, there are approximately 100 nuclei along the entire length of the A–P axis [1]. Gap gene circuits include 58 nuclei from $i = 35$ to $i = 92$, covering the region delimited by 35%–92% A–P position (where 0% is the anterior pole). This region corresponds roughly to the region of the fate map giving rise to thoracic and abdominal segments [2].

Pattern formation in the *Drosophila* blastoderm is a consequence of regulatory interactions among segmentation genes only. Mutations in segmentation genes affect expression of other segmentation genes, but do not cause any morphological phenotypes before gastrulation [3]. Therefore, the internal state of each blastoderm nucleus can be described by concentration levels of transcription factors encoded by segmentation genes. These protein concentration levels are the state variables of the model. We do not model RNA explicitly, since it has no known regulatory function in *Drosophila* segment determination. Gap gene circuits include the products of six zygotic genes denoted by index $a$: caudal (*cad*), hunchback (*hh*), Krüppel (*Kr*), giant (*gt*), knirps (*kni*) and tailless (*tll*). Maternal contributions to Cad and Hb are implemented as non-zero initial concentrations for the model at the onset of cycle 13. The exclusively maternal product of *bicoid* (*bcd*) is represented as an external regulatory input to zygotic gene expression.

1.2 Model: Rules and Equations

Gap gene circuit models have three rules governing the behaviour of nuclei in time:

1. interphase
2. mitosis
3. division.

Rules 1 and 2 are continuous and describe the dynamics of protein synthesis and decay within a nucleus, as well as protein diffusion between nuclei. Rule 3 is discrete and describes how each nucleus is replaced by its two daughter nuclei upon division. Rule 2 is necessary
since division is instantaneous in the model, while taking a certain amount of time in the
Drosophila embryo (see below).

The internal state of each nucleus \(i\) is described by a vector of transcription factor concentrations \(v_i\) where each element \(v_i^a\) corresponds to the concentration of a specific transcription factor encoded by gene \(a\). During interphase (rule 1), change in transcription factor concentration over time \(dv_i^a/dt\) depends on three processes:

1. regulated protein synthesis
2. protein diffusion between neighbouring nuclei
3. protein decay.

These three processes are represented by the three main terms on the right hand side of equation (1),

\[
\frac{dv_i^a}{dt} = R_a g \left( \sum_{b=1}^{N} T^{ab} v_i^b + m^a v_i^{Bcd} + h^a \right) + D^a(n) \left( (v_{i-1}^a - v_i^a) + (v_{i+1}^a - v_i^a) \right) - \lambda_a v_i^a,
\]

where \(N = 6\) is the number of zygotic genes in the model. A gap gene circuit consists of one ordinary differential equation (1) for each gene \(a\) in each nucleus \(i\). Since gap gene circuits include six zygotic genes and 58 nuclei, the total number of equations in the system during cycle 14A is 348.

In equation (1), \(T^{ab}\) represents a genetic interconnection matrix where each coefficient \(T^{ab}\) characterises the regulatory effect of the product of gene \(b\) (with concentration \(v_i^b\)) on the expression of gene \(a\). This matrix is independent of \(i\) reflecting the fact that each nucleus contains a copy of the same genome. The regulatory effect of Bcd (with concentration \(v_i^{Bcd}\)) on gene \(a\) is represented by the parameter \(m^a\). \(h^a\) is a threshold parameter representing regulatory contributions of uniformly expressed maternal transcription factors and determines the state of expression of gene \(a\) in the absence of Bcd and factors \(b\).

The relative rate of protein synthesis is given by the sigmoid regulation-expression function \(g(u^a) = \frac{1}{2} \left[ (u^a/\sqrt{(u^a)^2 + 1}) + 1 \right]\), where \(u^a = \sum_{b=1}^{N} T^{ab} v_i^b + m^a v_i^{Bcd} + h^a\) is the total regulatory input on gene \(a\). The maximum synthesis rate for the product of gene \(a\) is given by \(R^a\). The diffusion parameter \(D^a(n)\) depends on the number of nuclear divisions \(n\) that have taken place before the current time \(t\). Diffusion is assumed to vary inversely with the square distance between neighbouring nuclei and this distance is halved upon nuclear division. \(\lambda_a\) is the decay rate of the product of gene \(a\). It is related to the protein half life of the product of gene \(a\) by \(t_{1/2} = \ln 2/\lambda_a\).
During a mitotic division, chromatin is in a condensed state and hence inaccessible to transcription factors. Thus, during mitosis (rule 2) protein synthesis is shut down and only diffusion and decay occur,

$$\frac{dv_i^a}{dt} = D^a(n) \left[ (v_{i-1}^a - v_i^a) + (v_{i+1}^a - v_i^a) \right] - \lambda_a v_i^a. \tag{2}$$

At each division (rule 3), all nuclei divide instantaneously and equally and the distance between nuclei is halved. Protein concentrations in each daughter nucleus are identical to the concentrations in the mother nucleus. Note that as the number of nuclei doubles, the number of ordinary differential equations (1) in the system also doubles from 174 (during cycle 13) to 348 during cycle 14A.

1.3 Model: Time/Division Schedule

Gap gene circuits cover cleavage cycles 13 and 14A during the late syncytial blastoderm stage [1] including most of embryonic stages four and five in [2]. This covers the time between the first unambiguous detection of zygotically expressed Kr and Gt proteins in early cycle 13 [4, 5, 6], and the onset of gastrulation at the end of cycle 14A [1]. Time $t$ is measured in minutes from the onset of cleavage cycle 13 ($t = 0.0$ min). The time/division schedule for the model is based on [1] and shown in Fig. 5. Interphase (rule 1) of cycle 13 lasts for 16.0 min, mitosis (rule 2) from 16.0 to 21.1 min. All nuclei divide equally and simultaneously at the beginning of cycle 14A ($t = 21.1$ min). Interphase (rule 1) of cycle 14A then covers the remaining time from $t = 21.1$ min to the onset of gastrulation at $t = 71.1$ min.

Our quantitative gene expression data, is classified into one time class during cycle 13 and eight time classes during cycle 14A. Time points at half time through each class are used to compare model to expression data: C13, $t = 10.550$ min; T1, $t = 24.225$ min; T2, $t = 30.475$ min; T3, $t = 36.725$ min; T4, $t = 42.975$ min; T5, $t = 49.225$ min; T6, $t = 55.475$ min; T7, $t = 61.725$ min; T8, $t = 67.975$ min (Fig. 5).

1.4 Model: Bcd and Initial Conditions

Bcd is exclusively maternal. Therefore, regulatory input by Bcd is implemented as an external input represented by $m^a v_i^{Bcd}$ in equation (1). Bcd concentration $v_i^{Bcd}$ in each nucleus $i$ is assumed to be constant in time $t$ and is given by averaged quantitative Bcd expression data for time classes T1–T7 during cycle 14A (Fig. 6).

Kr, gt, kni and till are exclusively zygotic and we have not been able to detect their protein products before the onset of cycle 13 (data not shown). Therefore, initial conditions
Figure 5: Time/division schedule for gap gene circuits. The model spans the time from the onset of cycle 13 (0.0 min) to the onset of gastrulation at the end of cycle 14A (71.1 min). Rules 1–3 of the model (interphase, mitosis and nuclear division) are shown to the right. There is one time class in cycle 13 (C13), and eight time classes (T1–T8) in cycle 14A. Time points used for comparison of model output to data for each time class are indicated.
Figure 6: The Bcd gradient used in gap gene circuits. Bcd concentration is constant in time and is based on averaged Bcd expression data for time classes T1–T7. Grey background represents the region of the embryo included in gap gene circuit models.

Figure 7: Initial conditions for Hb and Cad represent maternal contributions to the expression of these proteins and are based on quantified expression data for cycle 12. Grey background represents the region of the embryo included in gap gene circuit models.
for these genes are zero. *hb* and *cad* have maternal and zygotic contributions. Maternal contributions are represented as non-zero initial conditions at the onset of cycle 13, based on quantified expression data for cycle 12 (Fig. 7).

1.5 Selection and Comparison of Gap Gene Circuits

Gap gene circuits obtained by Parallel Lam Simulated Annealing (PLSA) were selected as described in [7]. The selection process yielded ten gap gene circuits with diffusion (out of a total of 40). The quality of a circuit is reflected by its root mean square (rms) score, which is a measure for the average absolute distance between model output and quantitative gene expression data. All of the selected circuits have very similar rms scores [7] and show correct gap domain shifts (Fig. 8). Unless noted otherwise, graphs shown here and in the main paper are based on circuit 28008 which has an rms score of 10,170. This circuit shows no detectable patterning defects and its regulatory parameters correspond exactly to the gap gene network topology observed in a majority of the ten selected circuits [7]. Graphical dynamic analysis was performed on all ten circuits and variation in domain shift mechanisms between gap gene circuits are discussed below wherever applicable.

1.6 Gap Gene Circuit Parameters

Table 1 shows equation parameters for all gap gene circuits used in this study (see also Fig. 8). Note that parameters *h*<sup>hb</sup>, *h*<sup>Kr</sup>, *h*<sup>gt</sup>, and *h*<sup>kni</sup> were fixed to their respective negative values during optimisation, representing a constitutively repressed state for the corresponding genes. This increased annealing efficiency, while not affecting the quality of the resulting gap gene circuits. Circuit 31006 represents a gap gene circuit without diffusion. All its parameters *D*<sup>a</sup> were fixed to zero during optimisation. Circuit 33006 is a circuit without *kni* autoactivation and its parameter *T*<sup>*kni*—*kni</sup> was fixed to zero during optimisation.

2 Boundary Shift Analysis

2.1 The Posterior Boundary of the Posterior *kni* Domain

Based on Fig. 4, we propose the following dynamical mechanism for the shift of the posterior boundary of the posterior *kni* domain. *kni* autoactivation is only stably maintained in nuclei in the anterior part of the shift zone (Fig. 4i), whereas levels of Kni protein are kept low in the posterior part due to repression by Gt, Hb and Tll (Fig. 4j,k). While Tll repression
Figure 8: All ten selected gap gene circuits faithfully reproduce gap domain shifts. Time-space diagrams of rate of change in protein concentration $dv/dt$ for Kr, Kni and Gt are shown for all ten gap gene circuits used for graphical analysis. The vertical axes represent time, the horizontal axes % position along the A–P axis (where 0% is the anterior pole). Domains of protein synthesis are shown in yellow and bright red, domains of protein decay in black. Unless noted otherwise, all regulatory graphs shown below and in the main paper are based on circuit 28008 (red).
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Table 1: Equation parameters for gap gene circuits. $h_{bb}$, $h_{Kr}$, $h_{bt}$, and $h_{kni}$ were fixed to their respective negative values during optimisation. All $D^a$ parameters in circuit 31006, and $T^{kni-kni}$ were fixed to zero during optimisation. See text for parameter definitions.
decreases over time (Fig. 4k), repression by Gt (Fig. 4j) and Hb (Fig. 4k) increases during cycle 14A. Tll and Hb only affect the most posterior portion of the shift zone (Fig. 4k), Gt also acts more anteriorly (Fig. 4j). The repressive effect of Gt plays the crucial role of downregulating kni expression in regions where Kni protein is already present at a significant level (Fig. 4b,f,j). This is possible since gt is not significantly repressed by Kni (Fig. 4l), while kni is repressed by Gt (Fig. 4j). We have been able to detect this asymmetry in regulatory interactions between kni and gt in all circuits used for analysis, although repression of kni by Gt is very weak in circuit 29007 (Fig. 9a).

As shown elsewhere, the mechanism outlined above is consistent with experimental evidence [7]. The only exception to this is autoactivation of kni, which has never been found experimentally. Evidence from experiments with reporter constructs containing kni regulatory sequences even suggests autorepression of kni [8, 9]. In light of the above, we tested if kni autoactivation is necessary for the shift in the posterior kni domain in gap gene circuits. We obtained a gene circuit lacking kni autoactivation by optimisation with the corresponding regulatory parameter fixed to zero. This gene circuit (33006) has a very low rms score of 9.65, and shows correct boundary shifts for the posterior kni domain (data not shown). This suggests that kni autoactivation is not essential for the boundary shift.

Graphical analysis of circuit 33006 shows that in the absence of kni autoactivation, the shift of the posterior kni boundary is caused by a dynamical mechanism very similar to the one observed in other gap gene circuits, where kni autoactivation is present. Downregulation of kni in nuclei within the shift zone during cycle 14A contrasts with maintained Kni synthesis throughout most of that cycle in more anterior nuclei (Fig. 10a–f). In the absence of kni autoactivation, maintenance of kni expression in nuclei of the anterior portion of the shift zone is due exclusively to activating inputs by Bcd and Cad (Fig. 10g). This suggests that kni autoregulation can be replaced by activation through maternal gene products without altering the main dynamical characteristics of the boundary shift mechanism.

A non-essential role for kni autoactivation is further supported by analysis of the repressive interactions leading to downregulation of kni in nuclei within the shift zone. This downregulation is due to repression by Gt in the middle (Fig. 10h), and repression by Hb and Tll (Fig. 10i) in the posterior portion of the shift zone. Although the regulatory contribution of Gt is very small in circuit 33006, it provides the crucial regulatory input which lowers the relative synthesis rate below 10% (Fig. 10h). In accordance with other gap gene circuits, Gt expression in the region of the kni domain is made possible by the absence of gt repression by Kni (data not shown). Increasing repression of kni by Hb can only be observed in nuclei which show no expression of kni at all (Fig. 10i, see also Fig. 4k). This is because Kni is a
Figure 9: Plots of regulatory parameters in different gap gene circuits reveal posterior dominance in regulatory interactions between neighbouring gap genes. Plots are shown for regulatory parameters indicated in figure keys (see also Box 1 in the main paper). Background colour indicates activating parameter values ($\geq 0.005$, green), no interaction (parameter values between -0.005 and 0.005, yellow) or repression (values $\leq -0.005$, red) [7]. a, repression of \textit{kni} by Gt, but no effect on \textit{gt} by Kni, is observed in all circuits. Note that repression of \textit{kni} by Gt is very weak in circuit 29007. b, with the exception of circuit 28002, all circuits show repression of \textit{gt} by Hb, but activation of \textit{hb} by Gt. In circuit 28002, the absence of this regulatory asymmetry is compensated by exceptionally strong repression of \textit{gt} by Tll. c, only half of the circuits show repression of \textit{Kr} by Kni which is stronger than repression of \textit{kni} by \textit{Kr}. This suggests two alternative shift mechanisms for the anterior boundary of \textit{kni} in different circuits. See text for details. All regulatory graphs shown below and in the main paper are based on circuit 28008 (red) unless noted otherwise.
Figure 10: Graphical dynamic analysis of the shift in the posterior boundary of the posterior kni domain in circuit 33006 which lacks kni autoactivation. a–c, temporal behaviour of the rate of change in protein production $dv/dt$ and d–f, of the diffusion and the synthesis/decay terms of equation (1). Mitosis is shown as grey shaded background. The shift zone is limited by nuclei at 62 and 80% A–P position as described for Fig. 4. Nuclei within the shift zone (shown at 68% A–P position) show a characteristic switch from protein synthesis to decay during cycle 14A (arrow, b,e), whereas diffusion counteracts the boundary shift by generating an influx of protein into the zone of protein decay (asterisk, e). The inset in c shows positions of all three nuclei analysed here (grey lines) with respect to the temporal and spatial dynamics of the domains of Kni protein synthesis and decay (compare with Fig. 2b,e). g–i, temporal behaviour of regulatory contributions to kni expression. Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kr, Gt and Tll. Axes, dashed lines and definition of regulatory contributions as in Fig. 4.
very strong repressor of \( hb \) (Fig. 13f). Therefore, repression of \( kni \) by Hb is a consequence, rather than a cause of the \( kni \) boundary shift (see also Fig. 14).

In circuit 33006, repression of \( kni \) by Hb is very strong, whereas repression of \( kni \) by Gt is weak (Fig. 10h,i). In contrast, circuit 28008 shows strong repression of \( kni \) by Gt and weaker repression by Hb (Fig. 4j,k). Such variation in repressive strengths between circuits is not unusual. Four out of the ten gap gene circuits which have \( kni \) autoactivation also show very weak repression of \( kni \) by Gt (see Fig. 9a). This high variability of repressive strengths does not affect the qualitative similarity of the dynamic mechanisms causing posterior \( kni \) boundary shifts in different circuits with or without \( kni \) autoactivation. In all these circuits, Gt provides the essential repressive input to downregulate \( kni \) expression in the posterior portion of its posterior domain independent of how strong or weak this repression is (Fig. 4j,10h and data not shown). In summary, our analysis suggests that autoactivation of \( kni \) is not essential for the shift of the posterior \( kni \) boundary, and that the primary mechanism for the boundary shift is repression of \( kni \) by Gt, but not vice versa.

### 2.2 The Posterior Boundary of the Posterior \( gt \) Domain

The posterior boundary of the posterior \( gt \) domain shows the largest of all gap domain shifts during cycle 14A (Fig. 1e,f). It is caused by a mechanism whose dynamical principles are very similar to those underlying the posterior \( kni \) boundary shift, although it involves regulatory contributions by different regulators. In anterior nuclei of the shift zone, \( gt \) expression is maintained throughout most of cycle 14A (Fig. 11a,d) by a large, spatially uniform activating contribution by Cad with smaller activating contributions by Bcd and \( gt \) autoactivation (Fig. 11g). Within the shift zone, \( gt \) expression is downregulated, and the rate of change in protein concentration switches from synthesis to decay (Fig. 11b,e). More posteriorly, \( gt \) becomes repressed very early during cycle 14A (Fig. 11c,f). This is due to spatially specific repression by Hb in the central portion of the shift zone (Fig. 11h), as well as maintained repression by Tll more posteriorly (Fig. 11i). As in the case of the posterior \( kni \) domain, diffusion counteracts the boundary shift (Fig. 11e). As shown elsewhere, this dynamical mechanism is entirely consistent with existing experimental evidence [7].

As in the case of regulatory interactions between \( kni \) and \( gt \), we find a strong repressive posterior dominance for regulatory interactions between \( gt \) and \( hb \). With the exception of circuit 28002, \( gt \) is always repressed by Hb, while \( hb \) is weakly activated by Gt in most circuits (Fig. 9b). Graphical analysis suggests that this activating contribution is unlikely to be of functional significance by itself (data not shown). However, the absence of repression does allow activation of \( hb \) (see Fig. 14h) in the posterior portion of the \( gt \) domain which then
Figure 11: Graphical dynamic analysis of the shift in the posterior boundary of the posterior gt domain. a–c, temporal behaviour of the rate of change in protein production $dv/dt$ and d–f, of the diffusion and the synthesis/decay terms of equation (1). Mitosis is shown as grey shaded background. The shift zone is limited by a nucleus at 72% A–P position which does not show any protein decay at any time during cycle 14A, and the most posterior nucleus included in gap gene circuits at 92% A–P position. Nuclei within the zone (shown at 78% A–P position) show the same characteristic switch from protein synthesis to decay as do nuclei in the shift zone of the posterior kni boundary during cycle 14A (arrow, b,f). Diffusion counteracts the boundary shift by generating an influx of protein into the zone of protein decay (asterisk, e). The inset in c shows positions of all three nuclei analysed here (grey lines) with respect to the temporal and spatial dynamics of the domains of Gt protein synthesis and decay (compare with Fig. 2c,f). g–i, temporal behaviour of regulatory contributions to gt expression. Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kr, Gt and Tll. Kni does not repress gt. Axes, dashed lines and definition of regulatory contributions as in Fig. 4.
downregulates \( gt \) (Fig. 11h). In circuit 28002, the lack of repression by Hb is compensated by a very strong repression of \( gt \) by Tll. Since this circuit shows a slightly extended terminal posterior Tll domain [7], its unusual boundary shift mechanism can be considered an artifact of this particular circuit. Note that with the exception of circuit 28002, variation in repressive interactions involved in the posterior \( gt \) boundary shift is much smaller than in those involved in shifting the posterior \( kni \) boundary (Fig. 9a,b).

### 2.3 The Posterior Boundary of the Central \( Kr \) Domain

The shift of the posterior boundary of the central \( Kr \) domain is the smallest of the shifts in posterior gap domain boundaries analysed here (see Fig. 1e,f). It shows the same spatial combination of maintenance and downregulation of \( Kr \) synthesis as observed for the shifts in the posterior \( kni \) and \( gt \) boundaries (Fig. 12a–f). Once again, diffusion counteracts the boundary shift (Fig. 12e). Maintenance of \( Kr \) synthesis in anterior nuclei is caused by \( Kr \) autoactivation with additional activating contributions by Bcd and Cad (Fig. 12g). Within the shift zone, \( Kr \) becomes downregulated due to increasing repression by \( Kni \) (Fig. 12h). \( Kr \) is a very strong repressor of \( gt \) (see Fig. 13c). Therefore, only posterior nuclei with no \( Kr \) expression show strong repression of \( Kr \) by \( Gt \) (Fig. 12i). Surprisingly, gap gene circuits show two alternative regulatory mechanisms for the upregulation of \( kni \) within the \( Kr \) domain. These mechanisms are involved in the shift of the anterior \( kni \) boundary and will be discussed below (see Fig. 16).

### 2.4 The Posterior Boundaries of the Anterior \( hb \) and \( gt \) Domains

In contrast to the posterior boundaries of the central \( Kr \) domain and the posterior domains of \( kni \) and \( gt \), we were unable to detect significant shifts in the boundary positions of the anterior \( gt \) and \( hb \) domains in model and data (Fig. 13, see also Fig. 1). These boundaries sharpen and intensify during cycle 14A leading to a small decrease of residual \( Gt \) and \( Hb \) levels in more posterior regions of the boundary (Fig. 13a,b,d,e). Posterior shifts of these boundaries are prevented by strong repression of \( gt \) by \( Kr \) (Fig. 13c) and \( hb \) by \( Kni \) (Fig. 13f), which is consistent with existing experimental evidence [7].

### 2.5 The Anterior Boundary of the Posterior \( hb \) Domain

In the expression data, we have detected shifts in both boundaries of the posterior \( hb \) domain (data not shown). However, gap gene circuits show no shift in the peak or the posterior boundary of this domain. Elsewhere we show that the model gives incorrect results for
Figure 12: Graphical dynamic analysis of the shift in the posterior boundary of the central 
Kr domain. a–c, temporal behaviour of the rate of change in protein production $dv/dt$ and 
d–f, of the diffusion and the synthesis/decay terms of equation (1). Mitosis is shown as 
grey shaded background. The shift zone is limited by nuclei at 53 and 69% A–P position as 
described for Fig. 4. Nuclei within the zone (shown at 58% A–P position) show the same 
characteristic switch from protein synthesis to decay as do nuclei in the shift zone of the 
posterior kni boundary during cycle 14A (arrow, b,f). Diffusion counteracts the boundary 
shift by generating an influx of protein into the zone of protein decay (asterisk, e). The 
inset in c shows positions of all three nuclei analysed here (grey lines) with respect to the 
temporal and spatial dynamics of the domains of Kr protein synthesis and decay (compare 
with Fig. 2a,d). g–i, temporal behaviour of regulatory contributions to Kr expression. 
Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory 
contributions by Bcd, Cad, Hb, Kr, Kni, Gt and Tll. Axes, dashed lines and definition of 
regulatory contributions as in Fig. 4.
Figure 13: No significant boundary shifts are detected for the posterior boundaries of the anterior domains of *gt* and *hb*. **a,** **b,** **d,** **e,** comparison between *gt* (**a,** **d**) and *hb* (**b,** **e**) expression in expression data (**a,** **b**) and model output (**d,** **e**) at early (T1) and late (T8) cleavage cycle 14A. See Methods (in main paper) for time classes. Model output in **d,** **e** is based on circuit 28008 (red). **c,** **f,** plots of regulatory parameters reveal posterior dominance of repressive interactions involved in setting the posterior boundaries of the anterior *gt* and *hb* domain. Background colour indicates no interaction (parameter values between -0.005 and 0.005, yellow) or repression (values ≤ -0.005, red) [7]. **c,** repression of *gt* by Kr is always stronger than repression of *Kr* by Gt. **f,** repression of *hb* by Kni is always stronger than repression of *kni* by Hb.
activation of the posterior \( hb \) domain, most likely because we did not include the terminal gap gene \( hucklebein (hkb) \) in our current models \cite{7}. In contrast, the shift in the anterior boundary of the posterior \( hb \) domain is present in gap gene circuits (Fig 14a, inset).

Generally, shifts in anterior boundaries of gap genes are less extensive than those of posterior boundaries (see Fig. 1e,f). Moreover, all anterior boundaries are dependent on dynamical positioning of posterior boundaries of more anterior gap domains. In the case of the posterior \( hb \) domain, the shift in its anterior boundary is due to maintained expression of \( hb \) at moderate levels in nuclei within the shift zone (Fig. 14b,e). An additional contribution to the accumulation of Hb protein comes from influx of Hb protein by diffusion from more posterior nuclei (Fig. 14e). The upregulation of \( hb \) in nuclei within the shift zone is due to diminished repression by Kni (Fig. 14g–i). There is no repression of \( hb \) by Gt (see Fig. 9b). Therefore, the shift of the anterior boundary of posterior \( hb \) is a consequence of the shift of the posterior boundary of \( kni \) (see Fig. 4, 10). This mechanism is entirely consistent with available experimental evidence \cite{7}.

### 2.6 The Anterior Boundary of the Posterior \( gt \) Domain

The anterior boundary of the posterior \( gt \) domain (Fig. 15) is regulated by a mechanism very similar to the one for the shift in the anterior boundary of posterior \( hb \) (see Fig. 14). This shift essentially follows the shift of the posterior \( Kr \) boundary (see Fig. 12). Nuclei within the shift zone show moderate synthesis of Gt protein throughout cycle 14A (Fig. 15b,e). Moreover, there is influx of Gt protein into the shift zone, although this effect is transient and weaker than protein synthesis (Fig. 15e). In contrast, nuclei anterior of the shift zone remain strongly repressed by Kr throughout cycle 14A (Fig. 15a,d,g). More posterior nuclei show decreasing repression by Kr (Fig. 15h,i). There is no repression of \( gt \) by Kni (see Fig. 9a). This mechanism is consistent with existing experimental evidence \cite{7}.

### 2.7 The Anterior Boundary of the Posterior \( kni \) Domain

The situation is somewhat more complicated for the anterior boundaries of \( Kr \) and \( kni \). In the case of \( kni \), the main principles of boundary shift dynamics are very similar to those for the anterior boundaries of posterior \( hb \) and \( gt \). There is a zone of increased protein synthesis, as well as a zone of protein influx by diffusion (Fig. 16a,d,g), which contribute to increasing concentrations of Kni protein in the region immediately anterior of its expression domain. This dynamic behaviour is caused by two different regulatory mechanisms in different gap gene circuits. As an example, we compare these mechanisms between a circuit
Figure 14: Graphical dynamic analysis of the shift in the anterior boundary of the posterior \(hb\) domain. a–c, temporal behaviour of the rate of change in protein production \(dv/dt\) and d–f of the diffusion and the synthesis/decay terms of equation (1). Mitosis is shown as grey shaded background. The zone of the anterior boundary shift is limited by an anterior nucleus (at 69% A–P position) which shows no significant Hb protein synthesis (a), and a posterior nucleus (at 85% A–P position) which shows a reduction in Hb synthesis toward the end of cycle 14A (c). Nuclei within the shift zone (shown at 75% A–P position) show increasing synthesis of Hb protein at moderate levels throughout cycle 14A (arrows, e). Diffusion assists the anterior boundary shift by creating an influx of protein into the shift zone (asterisk, e). The inset in a shows positions of all three nuclei analysed here (grey lines) with respect to the temporal and spatial dynamics of the domains of Hb protein synthesis and decay. The shift of the anterior boundary of the posterior \(hb\) domain is clearly visible in the time-space diagram (inset, a). g–i, regulatory contributions to \(gt\) expression. Total regulatory input \(u\) is indicated by a black line. Red coloured areas represent the regulatory contribution by Kni, which is the only repressor of \(hb\) in our models. Axes, dashed lines and definition of regulatory contribution as in Fig. 4.
Figure 15: Graphical dynamic analysis of the shift in the anterior boundary of the posterior gt domain. a–c, temporal behaviour of the rate of change in protein production dv/dt and d–f of the diffusion and the synthesis/decay terms of equation (1). Mitosis is shown as grey shaded background. The zone of the anterior boundary shift is limited by an anterior nucleus (at 57% A–P position) which shows no significant Gt protein synthesis (a), and a posterior nucleus (at 72% A–P position), which corresponds to the anterior limit of the shift zone for the posterior boundary shift of gt and the zone of Gt protein decay (c). Nuclei within the shift zone (shown at 66% A–P position) show maintained synthesis of Gt protein at moderate levels throughout cycle 14A (arrows, e). Diffusion assists the anterior boundary shift by creating an influx of protein into the shift zone (asterisk, e). The inset in a shows positions of all three nuclei analysed here (grey lines) with respect to the temporal and spatial dynamics of the domains of Gt protein synthesis and decay (compare with Fig. 2c,f). g–i, regulatory contributions to gt expression. Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kr, Gt, Kni and Tll. Axes, dashed lines and definition of regulatory contributions as in Fig. 4.
with (Fig. 16b,e,h) and a circuit without (Fig. 16c,f,i) kni autoactivation. However, both mechanisms can be observed in different ‘wild type’ gap gene circuits with kni autoactivation (data not shown).

The first mechanism occurs in circuits which have significant repression of kni by Kr (Fig. 9c). In such circuits, repression of kni by Kr is overcome early during cycle 14A through activation by Cad (Fig. 16b). Later, the anterior shift is supported by anterior diffusion and subsequent increase of kni autoactivation during mid to late cycle 14A (Fig. 16d,e,g,h). All circuits with this mechanism have large kni autoactivation terms, and show activation of kni by Cad which is stronger than activation of Kr by Cad (data not shown). In contrast, circuits which do not have repression of kni by Kr show upregulation of kni due to decreasing repression by Hb (Fig. 16c,f,i). This is caused by the disappearance of Hb protein from central regions of the embryo as the posterior boundary of anterior hb sharpens during cycle 14A (see Fig. 13b,e). Comparison with experimental evidence favours the second mechanism, in which the shift of the anterior kni boundary is caused by decreasing repression by Hb. As mentioned above, there is no experimental evidence for kni autoactivation. The first mechanism relies on kni autoactivation while the second one does not, since it is present in a circuit without kni autoregulation. Moreover, there is no evidence for repression of kni by Kr, while repression of kni by Hb is supported by experiments, in which spatially specific misexpression of hb caused repression of kni expression in the affected regions of the embryo [10, 11, 12].

2.8 The Anterior Boundary of the Central Kr Domain

The shift of the anterior boundary of the central Kr domain is the smallest of all gap domain boundary shifts analysed here and, in our models, happens in a short period during early cleavage cycle 14A (Fig. 1). The position of the boundary is shifted by only two nuclei. This very subtle effect is caused by maintenance of moderate levels of Kr expression during cycle 14A in regions where Gt repression is not yet overwhelming (data not shown). Influx of Kr protein by diffusion into the shift zone also contributes to the shift. However, as the only anterior gap gene boundary analysed here, the anterior Kr boundary does not show any corresponding anterior shift in the anterior boundary of its domain of protein synthesis (Fig. 2a). Therefore, unlike other shifts of anterior boundaries, this shift is not caused by maintenance or upregulation of Kr expression in nuclei within the shift zone during cleavage cycle 14A.
Figure 16: Graphical dynamic analysis of the shift in the anterior boundary of the posterior kni domain. Spatial regulatory graphs are shown for early (T1, a–c), mid (T4, d–f) and late (T8, g–i) cleavage cycle 14A in a circuit with (28008, a,b,d,e,g,h) and a circuit without (33006, c,f,i) kni autoactivation. The inset in a shows the region covered and the time points of graphs shown in this figure (grey lines) with respect to the temporal and spatial dynamics of the domains of Kni protein synthesis and decay (compare with Fig. 2b,e). a,d,g, spatial profiles of the diffusion and the synthesis/decay terms of equation (1) showing a zone of protein influx by diffusion (asterisk) and an anterior shift in the peak of Kni synthesis (grey dotted line). b,c,e,f,h,i, spatial regulatory profiles of kni expression in circuit 28008 (b,e,h) and circuit 33006 (c,f,i). Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kr, Kni, Gt and Tll. Horizontal dashed lines and definitions of regulatory contributions as in Fig. 4. See Methods (in main paper) for time classes.
3 Gap Gene Circuits without Diffusion

We have shown in the main paper and above that diffusion is not involved in shifts of posterior gap domain boundaries, and plays an auxiliary role at best in the shift of anterior boundaries. A non-essential role for diffusion is further supported by results obtained from gap gene circuits which have no diffusion. Ten such circuits were obtained by fixing diffusion parameters $D_a$ to zero during optimisation (see Table 1). Two out of these ten circuits had rms scores of less than 12.0. The circuit with the lowest rms score (31006, rms = 11.507) was selected for graphical analysis. This circuit shows a gap gene network topology fully compatible with topologies found in circuits with diffusion [7]. Gap gene expression patterns in circuit 31006 show rugged instead of smooth spatial patterns and have more variability in the shape and steepness of boundary slopes than circuits with diffusion (Fig. 17a,e). However, positioning of gap domain boundaries relative to each other is correct and boundary shifts are clearly present in circuit 31006 (Fig. 17b–d, f), although these shifts occur in a more punctuated manner than in circuits with diffusion (compare Fig. 17 to Fig. 1c–h and 2a–c).

The regulatory mechanism underlying the shift in the posterior boundary of $kni$ in a circuit without diffusion is virtually indistinguishable from the mechanism found in other gap gene circuits (compare Fig. 18 with Fig. 10). Downregulation of $kni$ within the boundary zone (Fig. 18b,e) is provided by asymmetric repression of $kni$ by Gt (Fig. 18h), but not vice versa (data not shown). In more posterior nuclei, Tll and Hb retain $kni$ in a repressed state (Fig. 18i), while anterior nuclei show expression of $kni$ due to activation by Cad and $kni$ autoactivation in the absence of repression (Fig. 18g). Circuit 31006 also shows mechanisms for the shifts of the posterior boundaries of the central $Kr$ and the posterior $gt$ domain, which are very similar to those found in other circuits (data not shown). Taken together, this suggests that diffusion is not required for posterior boundary shifts at all.

In contrast to shifts of posterior boundaries, diffusion does contribute to shifts in anterior boundaries (Fig. 14–16). However, shifts in the anterior boundaries of the posterior $hb$, $gt$ and $kni$ domains can be detected in circuits without diffusion (Fig. 17 and data not shown). Graphical analysis of these shifts reveals differences in the dynamic regulatory mechanism of anterior boundary shifts in circuits with and without diffusion. For the shift of the anterior $kni$ boundary, circuits with diffusion show a combination of a shifting domain of protein synthesis and a domain of protein influx by diffusion anterior to the domain of synthesis (Fig. 16a,d,g). In contrast, circuit 31006 shows a mechanism which relies entirely on the maintenance of moderate amounts of Kni protein production during early and mid cycle 14A (Fig. 19a,c), reduced by increasing protein decay toward late cycle 14A (Fig 19e and data not shown). This sustained moderate synthesis of Kni in the shift zone is due to increasingly
Figure 17: A gap gene circuit without diffusion. a, e, model output of a gap gene circuit 31006, which was obtained by fixing all diffusion parameters $D^a$ to zero during optimisation. Model output is shown at early (a, T1) and late (e, T8) cleavage cycle 14A. Relative protein concentrations are shown for Hb, Kr, Kni, Gt and Tll. The region displayed covers 35–92% A–P position (where 0% is the anterior pole). c, gap domain shifts for $Kr$, $kni$ and $gt$ during the time between patterns shown in a and e. Solid dark coloured lines indicate position of maximum concentration for each domain. Lighter coloured areas cover regions in which protein concentration is above half maximum value. See Methods (in main paper) for time classes. b, d, f, time-space diagrams of rate of change in protein concentration $dv/dt$ for Kr (b), Kni (d) and Gt (f) based on model output from circuit 31006. The vertical axes represent time, the horizontal axes % position along the A–P axis. Note shifting positions of domains of protein synthesis (yellow, light red) and protein decay (black).
Figure 18: Graphical dynamic analysis of the shift in the posterior boundary of the posterior \textit{kni} domain in circuit 31006 which lacks diffusion. \textbf{a–c}, temporal behaviour of the rate of change in protein production \( \frac{dv}{dt} \) and \textbf{d–f}, of the synthesis/decay terms of equation (1). Mitosis is shown as grey shaded background. The shift zone is limited by nuclei at 62 and 80\% A–P position as described for Fig. 4. Nuclei within the zone (shown at 68\% A–P position) show the characteristic switch from protein synthesis to decay during cycle 14A (arrow, \textbf{b,e}). The inset in \textbf{c} shows positions of all three nuclei analysed here (grey lines) with respect to the temporal and spatial dynamics of the domains of \textit{Kni} protein synthesis and decay (compare with Fig. 2b,e). \textbf{g–i}, temporal behaviour of regulatory contributions to \textit{kni} expression. Total regulatory input \( u \) is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kni, Gt and Tll. Circuit 31006 has no repression of \textit{kni} by Kr. Axes, dashed lines and definition of regulatory contributions as in Fig. 4.
reduced repression of \(kni\) by \(Hb\) (Fig. 19b,d,f) caused by the sharpening of the posterior boundary of the anterior \(hb\) domain (Fig. 17a,e). This regulatory mechanism is very similar to mechanisms observed in gap gene circuits with diffusion (Fig. 16c,f,i). Moreover, the anterior boundaries of the posterior \(hb\) and \(gt\) domains still follow the posterior boundaries of \(kni\) and \(Kr\) in the absence of diffusion (Fig. 17c and data not shown). Only the very subtle shift of the anterior \(Kr\) boundary could not be observed in diffusion-less circuits (Fig. 17c).

In summary, we observe that in circuit without diffusion, shifts in anterior boundaries are caused by a slightly different dynamical mechanism than in circuits with diffusion. This mechanism relies entirely on upregulation of protein synthesis. However, the upregulation is due to the same regulatory mechanisms as in circuits with diffusion. Taken together, this suggests that with the exception of the anterior \(Kr\) boundary, diffusion is not required for regulatory mechanisms underlying shifts in anterior gap domain boundaries.

4 Summary of Shift Mechanisms

The results of our analysis are summarised in Fig. 20. In general, we see three different types of dynamical and regulatory behaviour of gap gene boundaries: (1) The posterior boundaries of anterior \(gt\) and \(hb\) sharpen and intensify, but do not show any boundary shifts. (2) The posterior boundaries of the central \(Kr\) domain and the posterior domains of \(kni\) and \(gt\) shift anteriorly due to repression by their immediate posterior neighbours, but not vice versa. (3) The anterior shifts of anterior boundaries follow shifts in posterior boundaries of more anterior gap domains. These three mechanisms together account for the observed narrowing and anterior shift of gap domains posterior of the anterior \(hb\) domain during cleavage cycle 14A (Fig 1e,f).
Figure 19: Graphical dynamic analysis of the shift in the anterior boundary of the posterior $\kni$ domain in circuit 31006 which lacks diffusion. Spatial regulatory graphs are shown for early (T1, a,b), mid (T4, c,d) and late (T8, e,f) cleavage cycle 14A. The inset in e shows the region covered and the time points of graphs shown in this figure (grey lines) with respect to the temporal and spatial dynamics of the domains of Kni protein synthesis and decay (compare with Fig. 2b,e of the main paper).  a,c,e, spatial profiles of the synthesis/decay term of equation (1) showing maintained protein synthesis at moderate levels throughout the shift zone in early and mid cycle 14A (arrows, a,c).  b,d,f, spatial regulatory profiles of $\kni$ expression in circuit 31006. Total regulatory input $u$ is indicated by a black line. Coloured areas represent regulatory contributions by Bcd, Cad, Hb, Kni, Gt and Tll. Circuit 31006 has no repression of $\kni$ by Kr. Horizontal dashed lines and definitions of regulatory contributions as in Fig. 4 of the main paper. See Methods (in main paper) for time classes.
Figure 20: Summary of mechanisms underlying dynamical behaviour of gap domain boundaries during cleavage cycle 14A. Expression patterns of *gt*, *hb*, *Kr*, *kni* and *til* are shown at late cycle 14A (T8) as in Fig. 1h. Dynamical and regulatory behaviour is summarised for posterior boundaries above, and for anterior boundaries below the expression graph. See text for details.
References


