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Precise domain specification in the developing *Drosophila* embryo.

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A simple morphogen gradient based on the protein *bicoid* is insufficient to explain the precise (i.e., similar in all embryos) setting of antero-posterior gene expression domains in the early *Drosophila* embryo. We present here an alternative model, based on quantitative data, which account for all of our observations. The model also explain the robustness of hunchback(*hb*) boundary setting in unnatural environments such as published recently [Luccheta et al., Nature, 434:1134-8(2005)]. The model is based on the existence of a secondary gradient correlated to *bcd* through protein degradation by the same agent.

I. INTRODUCTION.

The specification of gene expression domain in a developing embryo is the central problem of developmental biology. For *Drosophila Melanogaster*, the early events for the antero-posterior differentiation are well known: *bicoid* (*bcd*) mRNAs are deposited and localized by the mother at the anterior pole of the embryo. This localized source serves as a "fountain" for *bcd* proteins, which diffuse from there and are degraded throughout the embryo by some protease. The combined processes of diffusion from a source and degradation generate a stable, stationary (time independent) gradient of the *bicoid* protein with high concentration at the anterior and low concentration at the posterior pole. Downstream genes read the *bcd* concentration and set their boundaries according to different thresholds. The readout process can be direct, as in the case of hunchback or indirect, as for the gap genes which are activated by reading a combination of *bicoid* and hunchback (and each other). This is a cascading gene interaction network where *bcd* and *hb*, at the top, play a very special role. The general scheme for this kind of genetic network is called (simple) "morphogen gradient model"[1] (Fig.1(a-c)).

This picture has emerged over the past twenty years following several fundamental discoveries: (i) *Bcd* is a maternally active gene that encodes a transcription factor for many downstream genes expressed in the early embryo; (ii) *bcd* mRNAs are localized at the anterior pole of the embryo; (iii) *bcd* protein concentration profile forms a gradient across the embryo; (iv) modifying *bcd* dosage shifts downstream gene expression domains, in "agreement" with a threshold reading process[2, 3] (Fig.1(d)).

This simple morphogen gradient model suffers however from two important weaknesses: precision and scaling[4]. Precision here is to be understood as the degree of similarity between gene expression domains in different embryos. Scaling signifies the proportionality between gene expression domains and the embryo size.

**Precision.** The *bcd* concentration profile depends on various parameters such as the diffusion coefficient, *bcd* half life -which itself depends on the degrading agent concentration- and the quantity of mRNA deposited by the mother at the pole. Any embryo to embryo variation in these parameters will modify the *bcd* profile and thus affect the spatial extension of domains in these embryos. The problem with the simple morphogen gradient model is the lack of feedbacks and error correcting mechanisms. No usable machine however can be made without feedbacks.
Scaling. The shape of the bcd gradient is set by the above cited parameters, and none of them depends on the size of the embryo. This means that even if the mother were able to control precisely these parameters, the spatial extension of the hh domain will not be proportional to the embryo size.

As an example, if HB is activated at a Bcd concentration threshold normally found at 230 microns, that concentration will occur at the same average distance from the anterior end of the egg regardless of its length. In a 450 \( \mu \text{m} \) long embryo, \( hh \) domain will represent 51% embryo length (EL), whereas in a 500 \( \mu \text{m} \) embryo it will occupy only 46% EL. This “error” is equivalent to five nuclei. Any mechanism used to create the scaling has to sense the posterior end of the egg, and this is clearly lacking in a simple morphogen gradient. Of course, in the Drosophila embryo, there are known posterior gradients, such as \textit{nanos} which participates in the boundary setting of \( hh \), and \textit{caudal}, but we know that these genes do not have any role in the scaling of \( hh \).[4]

In our previous investigation [4], we have shown that indeed, \textit{bcd} is an extremely noisy signal and varies widely from embryo to embryo. The positional information \textit{bcd} can transfer to \textit{hb}, based solely on a threshold reading mechanism, has a standard deviation of 7% EL (approximately 7 nuclei). If the human nose were positioned arbitrarily 7 nuclei). If the mother provides another signal to the embryo, and if this second signal has the same source of fluctuation as that of \textit{bcd} (i.e. the two signals are correlated), then in principle the two noises can cancel out each other. We have previously shown that \textit{nanos}, or more generally, genes downstream of oscar, do not play the role of this secondary signal. Some alleles of the maternal gene staufen however disrupt the precision of the \( hh \) boundary, inducing fluctuation of the same magnitude as that of \textit{bcd}. Sta itself is not a transcription factor, but plays a role during oogenesis in the localization of anterior and posterior mRNAs[8].

In the reminder of this article, we will first investigate certain aspects of the \textit{bcd} gradient. We will then explore the error correcting capabilities of a (hypothetical) secondary signal. We will show that such a model is in extremely good agreement with ours and others observations.

II. THE BCD GRADIENT.

Let us revisit the establishment of the bicoid gradient. As mentioned above, \textit{bcd} proteins are produced at the anterior pole at a rate \( J \), diffuse through the embryo with a diffusion coefficient \( D \), and are degraded by some agent at a rate \( \omega \). The concentration \( B(x, t) \) of \textit{bcd} is given by the diffusion (Fick’s) equation \( \partial_t B = D \partial_x^2 B - \omega B \) with the boundary conditions \( \partial_x B|_{x=0} = -J \) and \( \partial_x B|_{x=L} = 0 \). \( L \) is the embryo length. The second boundary condition expresses the fact that \textit{bcd} molecules cannot cross the posterior extremity of the embryo. After a transitory time a stable, stationary (\( \partial B/\partial t = 0 \)) state is reached which obeys

\[
\frac{d^2 B}{dx^2} - \frac{1}{\lambda^2} B = 0 \tag{1}
\]

where \( \lambda = \sqrt{D/\omega} \) is the diffusion length, i.e. the average distance a molecule diffuses before degradation. The stationary solution reads:

\[
B(x) = C_1 \exp(-x/\lambda) + C_2 \exp(+x/\lambda)
\]

The amplitudes are \( C_1 = \frac{J\lambda}{(1 - \exp(-2L/\lambda))} \) and \( C_2 = \exp(-2L/\lambda)C_1 \). For \textit{bcd} gradient in Drosophila, the average diffusion length is \( \lambda = 0.26L \). We can thus drop the positive exponential and approximate the gradient by

\[
B(x) \approx (J\lambda) \exp(-x/\lambda)
\]

The error in the approximation is 0.03% at the anterior and 2% at the posterior pole.

The measurement of the \textit{bcd} gradient is most conveniently achieved by immuno-fluorescence staining techniques : in each embryo the local intensity of fluorescence
The profile of the bcd gradient in a bcd6X embryo measured by immuno-fluorescence. The fluorescence intensity data (black) is fitted to an exponential (red) $a \exp(-x/\lambda) + b$.

$I(x)$ staining is extracted by image analysis techniques (see Material and Methods). Figure 2 displays such a measurement in one embryo.

In order to assess embryo to embryo variability, the most robust parameter to measure is the diffusion length $\lambda$, which is independent of the fluorescent staining noise and can be obtained by directly fitting the function $a \exp(-x/\lambda) + b$ to $I(x)$. As reported before, the embryo to embryo variability of the diffusion length is $\delta \lambda = 0.05$ EL. It follows that if Hb was activated directly by bcd, the embryo to embryo variability of its boundary would be $\delta x_{3ab} = 0.07$ (approximately seven nuclei).

There exists a possibility for this signal to be less noisy than it appears, at least for Hb activation at mid-embryo: If in an embryo, the bcd-degrading agent has a higher concentration, then $\lambda$ is smaller and a smaller proportion of molecules produced at the anterior pole reach the mid embryo; if however, in the same embryo, more Bcd mRNA is deposited at the pole, the synthesis rate at the origin would be higher. The combination of these two effects can induce the same number of bcd molecules to reach the middle of the embryo. Individual absolute amplitudes (Fig.2) cannot be measured directly from the images, because of the additional uncertainty induced by fluorescent staining, but if the above hypothesis were true, we should observe a negative correlation between the amplitude $a$ of the fitted signal and its slope $\lambda$. No significant correlation has been found however: on $N = 91$ samples analysed, the spearman correlation coefficient obtained was $r_{sp} = -0.09$ ($P = 0.4$).

As we mentioned above, individual signal amplitudes cannot be measured for embryos. It is possible however to compare the average signal amplitudes in different backgrounds if embryos are stained at the same time in the same conditions. Then, the experimental error induced by staining is similar for all embryos and by averaging the amplitudes over enough embryos in the same background, a good estimation of the signal strength in one background compared to the other can be obtained (see Material and Methods).

### Table I: Average Relative (to WT) amplitude of the bcd gradient in embryos derived from mothers with variable numbers of Bcd transgenes.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Rel. Ampl.</th>
<th>$\lambda$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>29C</td>
<td>0.80 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>32</td>
</tr>
<tr>
<td>25C</td>
<td>1.0 ± 0.04</td>
<td>0.26 ± 0.04</td>
<td>19</td>
</tr>
<tr>
<td>18C</td>
<td>0.84 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>24</td>
</tr>
<tr>
<td>9C</td>
<td>0.68 ± 0.06</td>
<td>0.80 ± 0.05</td>
<td>15</td>
</tr>
</tbody>
</table>

### Table II: Average bcd gradient amplitude at various temperatures, and its exponential decay length (%EL). Amplitudes are relative to 25C condition.

<table>
<thead>
<tr>
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III. SECOND MORPHOGEN HYPOTHESIS.

In the simplest model of antero-posterior specification, Hb is activated when the bcd signal is above a given threshold $c_0$. In this model, $hb$ boundary would be as (embryo to embryo) variable as $bcd$. As we mentioned above, the $bcd$ error can be corrected maternally if a second morphogen were present in the embryo and its fluctuations correlated to that of $bcd$. Then, the errors of these two signals can cancel each other. Let us again insist that even though nanos participates in the $hb$ boundary setting (indirectly, by degrading maternal $hb$ mRNA), it is not the second morphogen considered here: its removal (with or without the maternal $hb$) does not affect the precision of $hb$.

The $bcd$ variability we measure is in fact the variability of its exponential decay length $\lambda$ (which has a standard deviation of 0.05 EL). The decay length in turn depends on the diffusion coefficient $D$ of $bcd$ molecules and the
degradation rate \( \omega \). The diffusion coefficient, a passive parameter which is related to viscosity should not vary from embryo to embryo. On the other hand, the degradation rate depends on the quantity of degrading agents the mother deposit in the embryo which can be highly variable and the main source of fluctuations in \( bcd \) gradient. In order for the second morphogen to be correlated to \( bcd \), it will be enough for it to be degraded by the same agent which degrades \( bcd \). Then, in a given embryo, if \( \omega \) is higher (lower) than average, both morphogens will have a smaller (bigger) diffusion length.

The other fact we mentioned is the scaling property of \( hb \) boundary. Whatever the activation mechanism, it has to be influenced by the distance to both poles\([9]\). \( bcd \) has a vanishing value at the posterior pole and cannot play such a role.

The simplest model of a second morphogen (which we will designate by the letter \( P \)) taking into account the above ideas is the following: (i) \( P \) is produced from a localized source of mRNA at the posterior pole, diffuses and is degraded with the same rates as \( bcd \), so it makes an exponential gradient going from high values at the posterior to small values at the anterior; (ii) it is an inhibitor of Hb. More precisely, Hb is activated in the region where \( B(x) > P(x) \), so the boundary position of \( hb \) is given by the condition (Fig. 3):

\[
B(x_{hb}) = P(x_{hb}) \quad (2)
\]

This condition is easily realised for example if \( bcd \) and \( P \) compete for the same sites on the regulatory region of the Hb gene. In principle, we should speak about the activities of these two proteins and not their actual concentration. \( P \) can have twice the activity of \( bcd \) and be present at half the concentration. Without loss of generality however, and to keep the model as simple as possible, we will use concentrations instead of activities.

Repeating the arguments of the introduction, \( P(x) \) obeys the diffusion equation \( d^2P/dx^2 - \lambda^{-2}P = 0 \) with the boundary conditions \( dP/dx|_{x=0} = 0 \) and \( dP/dx|_{x=L} = J \) (production at the posterior pole). Thus \( P(x) \) reads (Fig. 3)

\[
P(x) \approx (\lambda x) \exp \left( \frac{x - L}{\lambda} \right) \quad (3)
\]

where in each embryo, the diffusion length for both morphogens are the same. This hypothesis is a consequence of \( P \) being degraded by the same agent as \( bcd \). Let us now consider the plausibility of this model and its many prediction.

Correcting for errors and scaling. If Hb was activated only by \( bcd \) through a thresholding mechanism \( B(x_{hb}) = c_0 \), its embryo to embryo fluctuation would be \( \delta x_{hb} = -\delta \ln c_0 = 0.07 \) (in EL units). If however Hb was activated by two gradients as explained above, the condition (2) reads

\[
\exp(-x_{hb}/\lambda) = \exp \left( \frac{x_{hb} - L}{\lambda} \right)
\]

and thus the \( hb \) boundary is given by (see Fig. 3)

\[
x_{hb} = L/2
\]

We have here two remarkable facts. First, the position of \( hb \) is set independently of \( \lambda \), the source of fluctuations: The errors in the two gradients have cancelled out each other. Second, the \( hb \) position is automatically proportional to the embryo size \( L \). This simple model explains two of the elusive behaviors of Hb activation.

Let us also note that by this mechanism, when both posterior and anterior gradient have the same slope, error correction is optimal at mid-embryo, precisely where \( hb \) boundary is actually set. In principle, the two gradients mechanism can set the boundary at any position if the condition for the gene activation were \( uB(x_{hb}) = P(x_{hb}) \). Then, the position of \( hb \) boundary would be

\[
x_{hb} = \lambda (\ln u)/2 + L/2
\]

But this would not be proportional to the embryo size \( L \). Even worst, fluctuations in \( \lambda \) would not be corrected any more and

\[
\delta x_{hb} = \delta \lambda (\ln u)/2 \quad (4)
\]

In the general case, anterior and posterior gradients can have different slope, and the position of a given gene’s boundary will be given by \( \lambda_1 \lambda_2 (L + \lambda_2 \ln u) \).

Again, scaling can be achieved only if \( u = 1 \). Moreover, if we suppose the embryo to embryo fluctuations of slopes \( \delta \lambda_1 \) to be due to the degrading agent concentration fluctuations \( \delta C \) and thus \( \delta \lambda_1/\lambda_1 = (1/2)\delta C/C \), then

\[
\delta x = \left( \frac{\lambda_1 \ln u}{\lambda_1 + \lambda_2} \right) \delta \lambda_2
\]
and the error is corrected again only if $u = 1$.

A last issue is error correction for amplitude variations. There exists a priori an embryo to embryo variation in the quantity of mRNA deposited by the mother which we have neglected in the above discussion. This source of fluctuation - which cannot be measured by fluorescent staining and is independent of variation in diffusion length - would add to errors in $hb$ boundary. This error also can be corrected by the two gradients mechanism, if there is correlation between mRNAs of localized posterior and anterior morphogens, i.e. the same quantity is deposited at both poles. It is probably no coincidence that the only gene which disruptions the precision of $hb$ is one that is responsible for mRNA localisation at both poles.

Effect of bed gene copies. The most convincing argument for bicoid being indeed a morphogen was given by Driever and Nusslein-Wolhard in 1988 when they showed that providing more or less copies of Bcd gene to the embryo by the mother shifts the position of downstream genes toward anterior or posterior in the embryo[3]. This argument however does not hold quantitatively if $bcd$ were the sole morphogen. By modifying the number of genes in the mother, the amplitude of $bcd$ in derived embryos becomes $u$ times higher than in WT ($u = 1$ for WT). Then the condition $B(x_{hb}) = u \exp(-x/\lambda) = c_0$ of the simple morphogen gradient model implies that the expected shift (compared to WT) in average $hb$ position be $\Delta x_{hb} = \lambda \ln u$. In a two gradients model however, the expected shift in $hb$ boundary will be only half of this value : $\Delta x_{hb} = (\lambda/2) \ln u$ (Fig.4). Table III shows the comparison between measured shifts in embryos with various background and values expected from simple and two gradients model. As it can be observed, only the two gradients model is in agreement with the measurement. Note that if we had assumed a normal activity for transgenes ($u = 2$ for $bcd4X$ and $u = 3$ for $bcd6X$), the discrepancy of the simple morphogen model would be much higher, but the two gradients model predictions will still be acceptable.

Temperature compensation. As showed in table II $bcd$ amplitude and diffusion length are function of temperature. There is no reason a priori for a simple morphogen gradient to be temperature compensated, i.e. specify the same boundary position for $hb$ whatever the temperature. Without more knowledge of the detailed activation rates however, this possibility cannot be ruled out, at least for the activation of a single gene : the $Hb$ activation threshold can vary in such a manner as to compensate the variation in the other two parameters. This however remains a fragile process with no feed back. A two gradients mechanism on the other hand corrects naturally for temperature variations : if for example $bcd$ and $P$ compete for the same regulatory region, they are affected in a similar way by temperature variation and the condition $B(x_{hb}) = P(x_{hb})$ remains valid at all temperatures.

Precision in a non-uniform temperature gradient. Using a microfluidics device, Luccheta et al. have been able to keep one half of an embryo at one temperature (18C) and the other half at another temperature (25C)[10]. Even though development time is highly different in the two halves, the $hb$ boundary is still set at mid-embryo with high precision. As we will show below, This is what a two gradients model predicts. The nature of this regulation is summarized in Fig.5 , where the posterior half is maintained at 18C and the anterior half at 25C. The source (mRNAs) for the posterior gradient being at 18C, synthesis of $P$ is reduced at the posterior pole. The lower temperature in the posterior half of the embryo however induce also an increase in the diffusion length in this part. As a consequence the number of $P$ molecules reaching the mid-embryo are the same that the number of $bcd$ molecule coming from the anterior pole.

More precisely, a variation in temperature affects the synthesis rate $J$ and the diffusion length $\lambda$ for which we possess quantitative data. The diffusion equation for $bcd$ reads in this non-uniform temperature gradient

$$\frac{d^2 B}{dx^2} - \frac{1}{\lambda(x)} B(x) = 0$$

with $\lambda(x) = \lambda_1$ if $x < L/2$ and $\lambda(x) = \lambda_2$ if $x > L/2$, where subscript 1 and 2 refer to diffusion length in the anterior and posterior halves of the embryo, set by temperature in these parts. Two of the boundary conditions read as before $d^2 B/dx^2|_{x=0} = -J_1$ and $d^2 B/dx^2|_{x=L} = 0$. $J_1$

<table>
<thead>
<tr>
<th>Background</th>
<th>$u$</th>
<th>$u$ measured</th>
<th>Simple grad.</th>
<th>Two grads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcd1X</td>
<td>0.5</td>
<td>-0.08 ± 0.01</td>
<td>-0.18</td>
<td>-0.09</td>
</tr>
<tr>
<td>Bcd4X</td>
<td>1.6</td>
<td>0.07 ± 0.015</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Bcd6X</td>
<td>1.9</td>
<td>0.10 ± 0.02</td>
<td>0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table III: Measured shift in $hb$ boundary (relative to its position in WT) compared to predictions of simple and two gradients model. The quantity $u$ denotes the strength of $bcd$ amplitude in different genetic background. $Bcd$ amplitudes in mothers with transgenes have been measured (table I).
The simple morphogen model, where \textit{bcd}, in a concentration dependent manner, specifies gene expression do-
mains lacks feedback mechanisms and cannot quantita-
ively account for many phenomena. These phenomena
include high precision and scaling properties of down-
stream genes; smaller than expected shift when the am-
plitude of \textit{bcd} is changed; temperature compensation,
specialy when embryos are maintained in non-uniform
temperature field.

We have shown in this article that all these phenom-
ena can be accounted for if we suppose the existence of a
second posterior morphogen correlated to \textit{bcd}. The cor-
rection mechanism in this “two gradients model” is based
on a simple principle : if a signal is noisy, duplicate it
by taking its mirror image and subtract the second from
the first. Then, at one position inside the embryo (the
mid-embryo being the optimum choice), the noises of the
two gradients cancel each other completely. This is where
the \textit{hb} boundary is set and this precise signal can then be
transmitted to downstream genes. It is remarkable how
such a simple model can explain so many different obser-
ations, either obtained by us or recently by Luccheta \textit{et
al.}

The second gradient remains however a hypothesis and
it would seem surprising that more than 20 years after the
genome wide screen there are still genes not uncovered.
Until (and if) the second morphogen is found, the “two
gradients” model is only a plausible framework, similar to
the “simple gradient” model until 1987 and the discovery
of \textit{bcd}. It is however significant that the only mutation
we have found which disrupt the precision of boundary
setting is the \textit{maternal} gene Stauffen, which is respon-
sible for localizing mRNAs at both anterior and posterior
poles. More work is needed at this level to understand the
nature of molecular events caused by Stauffen mutation.

We believe however that the exact mechanism cannot be
very different from the general scheme we have presented
here.

\textbf{Acknowledgement.} We are grateful to A. Nouri,
G. Dolino and M. Vallade for thoughtful discussions and
critical reading of the manuscript.

Appendix A: Material and Methods

1. Staining and Image analysis.

Embryos were collected at cycle 14 and immunostained
following published protocols[11], except for the final
rinsing time which is an important step in reducing non-
specific antibodies attachment. The best results were
obtained by 3 days rinsing (signal to noise ratio of \approx 20
can be obtained). Most results were obtained by 1 day
rinsing (S/N \approx 10). When studying temperature effects,
embryos were collected for 1h at 25C and then allowed to
reach cycle 14 at the set temperature (20h at 9C). Anti-
obodies were a gift of J. Reinitz and David Kossman[12].
High resolution (1317×1015pixels, 12bits/pixel) images of
stained embryos in a given condition were taken. Im-
ages were focused at mid-embryo to avoid geometric dis-

Figure 5: "Color online" Anterior and posterior gradients for
the setting of \textit{hb} boundary in a non-uniform temperature gra-
dient. The anterior half of the embryo is maintained at 25C and
its posterior half at 18. Values for synthesis rates and
diffusion lengths are set accordingly to Table II. The position
\(B(x_{hb}) = P(x_{hb})\) is marked by a circle. For comparison, the
two gradients are also shown in a uniform temperature field.
Concentrations (y-axis) are relative to uniform temperature
25C.

is the synthesis rate of \textit{bcd} at the anterior pole set by local
temperature there. There are two additional conditions
of continuity of concentration and flux at the bound-
dary between high and low temperature : \(B(L/2^-) =
B(L/2^+)\) and \(dB/dx|_{x=L/2^-} = dB/dx|_{x=L/2^+}\). The so-
}
tortions. Intensity profiles were extracted by sliding a rectangle, the size of a nucleus, along the inner edge of the embryo, itself detected by intensity thresholding. The average was computed on the brightest half pixels of the rectangle in order to compensate for the space between nuclei. The coordinates of the rectangle were projected on the main axes of the embryo, and the intensity \( I(x) \) recorded separately for dorsal and ventral side.

2. Amplitude quantification.

The \( B_i \) concentration \( B_i(x) \) in the embryos \( i \) reaches a high value at \( x^*_i \) which we call its amplitude \( A_i \) (Fig. 2) and drops to vanishing level at the posterior pole \( B_i(L) \approx 0 \). In the fluorescent signal we measure in this embryo, a part \( \beta_i \) is due to non-specific antibody binding and another part \( \alpha_i \) to specific ones. Therefore, the fluorescent intensity in this embryo reads

\[
I_i(x) = \alpha_i B_i(x) + \beta_i
\]

\( \alpha, \beta \) and \( B(x) \) are random variables (varying from embryo to embryo) with given averages and standard deviations. If all embryos from various background are stained at the same time in the same conditions, then \( \alpha \) and \( \beta \) have the same distribution for all embryos and depend only on staining conditions. \( B(x) \) on the other hand depends only on the genetic background of the embryos. Using the exponential variation of \( bcd \) in each embryo, the term \( \beta_i \) can be evaluated as \( I_i(L) \). Therefore the measured quantity we call “signal” \( S_i = I_i(x^*_i) - I_i(L) = \alpha_i A_i \). Averages over all embryos in a given background, and assuming independence of \( \alpha \) and \( A \):

\[
\langle S \rangle_{\text{bckgrnd1}} = \langle \alpha \rangle_{\text{bckgrnd1}} \langle A \rangle_{\text{bckgrnd1}}
\]

To compare the relative amplitude of \( bcd \) in two different backgrounds, one has only to evaluate the ratio of the average signals in these two backgrounds:

\[
\frac{\langle A \rangle_{\text{bckgrnd1}}}{\langle A \rangle_{\text{bckgrnd2}}} = \frac{\langle S \rangle_{\text{bckgrnd1}}}{\langle S \rangle_{\text{bckgrnd2}}}
\]

In order to decide if the differences in amplitudes are significant, note that \( \delta A / A = \delta S / S - \delta \alpha / \alpha < \delta S / S \). Therefore, if the differences in measured average signals \( \langle S \rangle \) are significantly different (from a statistical point of view), then so are the estimated amplitudes \( \langle A \rangle \). The best indicator for the significance of the random variable \( \langle S \rangle \) measured is its standard error, i.e. the standard deviation of the random variable \( S \) divided by the square root of the number of samples. The significance of differences can be further evaluated by a Student’s \( t \)-test.

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[5] Struhl G., Johnston P. & Lawrence PA., control of Drosophila body pattern by the hunchback morphogen gradient, Cell 69:237-240 (1992). Note that for the authors, \( hh \) is a complement to \( bcd \), not an “error corrected” morphogen.