Filtering transcriptional noise during development: concepts and mechanisms

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Abstract | The assignation of cell fates during eukaryotic development relies on the coordinated and stable expression of cohorts of genes within cell populations. The precise and reproducible nature of this process is remarkable given that, at the single-cell level, the transcription of individual genes is associated with noise — random molecular fluctuations that create variability in the levels of gene expression within a cell population. Here we consider the implications of transcriptional noise for development and suggest the existence of molecular devices that are dedicated to filtering noise. On the basis of existing evidence, we propose that one such mechanism might depend on the Wnt signalling pathway.

Cell state
The molecular phenotype of a cell. At the transcriptional level, it is defined by the profile of gene expression that defines its phenotype. It can refer to a terminally differentiated cell or to one that is at a certain stage of development.

Non-Markovian sequence
A sequence of events in which a given step depends exclusively on the position of the system and not on its history. One example is the fate of adult stem cells, which can be coaxed to differentiate into any cell type independently of their history.

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The development of multicellular organisms relies on generating different types of cell and assigning to them different shapes and functions[1–3]. The number of different types of cell can be as high as 10^{14} in mammals, which includes ~10^{9} different types of neuron that make up the cerebellum alone. This number is probably orders of magnitude higher when we take into account the number of transitional states (the cell states that lead from a naive cell in an early embryo, say at the blastoderm stage, to a differentiated type such as muscle), the number of cells that are renewed every day in the haematopoietic system, skin or intestine, and the different physiological states of a given cell. At the most basic level, a particular cell state is defined by a gene-expression profile and therefore by the set of transcription factors that determine its phenotype. As the number of these factors in an organism is smaller than the number of types of cell that they have to specify, and usually no more than 20% of the genome's coding capacity[4–7], it is no surprise that these factors act in combinatorial codes. Development is, in essence, a sequence of transitions between cell states that is governed by a combination of intrinsic influences (the transcriptional history of a cell) and extrinsic influences (intercellular signals), which determine a transcriptional code for each cell.

A sequence of cell-fate transitions can be represented by a non-Markovian sequence of simple binary choices. At each choice-point cells face a decision between two alternative gene-expression profiles that will determine their 'state' and the developmental potential that it represents (FIG. 1). Even the response of a cell to a morphogen[2,8] can be construed in this manner because at any given position within a field, a cell that is exposed to a morphogen is taking a binary decision between remaining in the 'ground' state or expressing the genes that are determined by the local concentration of the morphogen (FIG. 1b).

The large number of cell states that are present in the lifetime of an organism and the reproducibility with which they are generated indicates the existence not just of programmes but also of mechanisms that ensure their reliable execution. This is particularly obvious when we take into account the existence of noise — fluctuations in the performance of molecular operations that create variability in the phenotype of a cell population. However, in the context of development, particularly pattern formation, this variability opposes the precise and reproducible behaviour that is observed in cell populations. If, as it seems, noise is an intrinsic property of biological systems the question arises as to how it is controlled and whether it can be used in the construction of an organism[9].

Here we begin to explore these issues in the context of embryonic development, on the basis of information that has been gathered from recent studies in bacteria and yeast. We first introduce the conceptual and experimental framework for studying noise that is being laid down by these studies; we then focus on the process of cell-fate assignation and propose a general scheme that could be used as a framework to analyse the influence of noise in this process. Within this context we surmise that developmental systems have built-in molecular systems that control noise, and present some evidence that the Wingless/INT (Wnt) signalling pathway might be one such device.

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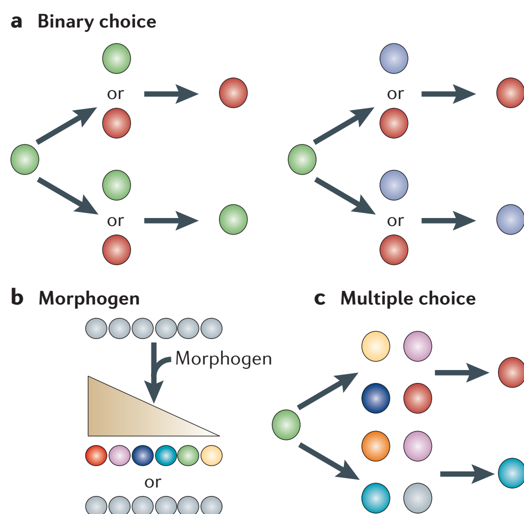

Figure 1 | **Different modes of cell-fate assignation during development. a** | After division, the two daughter cells often face a choice of fates. In some instances (left) one of the daughters remains in the initial state and the other adopts a new state. This is a situation that is common in stem cells. In other instances (right), each of the two daughters adopts one of two fates, both of which are different from that of the precursor. This is a situation that is characteristic, for example, of many lineages in the development of the nervous system. In both these situations the choice is binary. **b** | Morphogens determine cell fates in a naive cell population in a concentration-dependent manner. At each point along the gradient a cell has a binary choice between remaining in the ground state or acquiring the state that is determined by the local morphogen concentration. **c** | One exception to this binary-choice concept is represented by the lineages of the haematopoietic system, wherein cell fate seems to follow a multiple-choice pattern: precursors can initially express many of the features of different sublineages[90] and even other tissues[91], but are then reduced to one fate by repression of the others.

Morphogen
A signalling molecule that elicits sensitive concentration-dependent responses in gene expression.

Gene-regulatory networks
Functionally significant arrangements of regulatory interactions between individual genes that carry out specific information-processing tasks.

Fidelity
The reliability with which a process is executed; the degree of reliability between the input and output of a pathway or network.

Transfer function
A parameter that measures the relationship between the input and output in a network.

Sources of noise that affect gene expression

Transitions between cell states during development are driven by a combination of transcription and translation, that is, by controlled interactions between DNA, RNA and proteins. Like any molecular interaction these transitions are subject to random fluctuations that can influence their outcome — in other words, they have an inherent level of noise (BOX 1). Recent work on bacteria and yeast demonstrates that noise is an important component of gene expression[9–14]. The sources of noise that are associated with gene expression include the small number of molecules that are involved in a particular biochemical reaction in the cellular space, the stochastic nature of the molecular interactions that underlie the chemical reaction, and the built-in efficiency of the transcriptional and translational processes. Experiments on simple engineered gene-regulatory networks (GRNs) have begun to tease apart the contribution of each of these elements to the fidelity of gene expression in single cells and in cell populations[11–14] (BOX 1).

Most studies of the noise that is associated with gene expression have focused on transcription, and have distinguished between noise that is derived from the interaction between polymerases and DNA (intrinsic noise) and that which is produced by other influences such as random fluctuations in nutrients, cell division or regulatory inputs to the transcriptional machinery (extrinsic noise)[11,13] (BOX 1). These experiments have revealed that noise is a pervasive property of GRNs, is gene-specific and occurs in translation as well as in transcription[10,11,14].

An important difference between transcription in prokaryotes and eukaryotes is the existence of chromatin in eukaryotes and the requirement to regulate its structure to achieve effective gene expression. The significance of this difference for the analysis and modelling of noise is still uncertain. However, in *Saccharomyces cerevisiae* the assembly of transcriptionally competent chromatin is a source of noise and a target for its regulation[12]. This study revealed that whereas mutations in the TATA box upstream of the repressible acid phosphatase *PHO5* do not significantly change the level of noise that is associated with *PHO5* expression, mutations in the upstream activating sequences (UASs) do change it. This observation implicates chromatin remodelling as a noise-sensitive component of transcription. In support of this conclusion, mutations in the genes (*SNF6*, *ARP8*, *GCN5*), which encode the components of the chromatin-remodelling complex that acts on UASs, have an effect on the levels of noise that is similar to that of mutations in the UASs themselves[12].

The significance of chromatin remodelling for gene expression[15,16], and the possibility that it is a source of noise during transcription, indicates that it is important to determine the level of noise that is associated with gene expression not only in isolated single cells, but also in tissues and organs. Furthermore, it is important to establish how the noise that is associated with a particular transcriptional event is related to other linked events as part of a pathway or network.

Noise in gene-regulatory networks

Network architecture and noise sensitivity. Because the output of GRNs determines the structure and fitness of an organism[17], it is important to evaluate how the noise that is associated with gene expression affects GRN performance. This question has begun to be addressed by analysing the performance of engineered GRNs in bacteria and, to a less accurate degree, in yeast. In these studies linear networks are designed in such a way that each step can be controlled and monitored; the experimental data are then contrasted with predictions made through modelling approaches, which helps to determine the sensitive parameters of the system[10,14,18–21]. Of particular value in these studies is the transfer function of a network, which determines how well a system performs. These experiments show that the system responds more sharply as the number of links in a network increases, that is, the system becomes more sensitive to a threshold value of the input and has a more precise information-processing capacity. However, noise is transferred from one step to the next and has a tendency to be amplified

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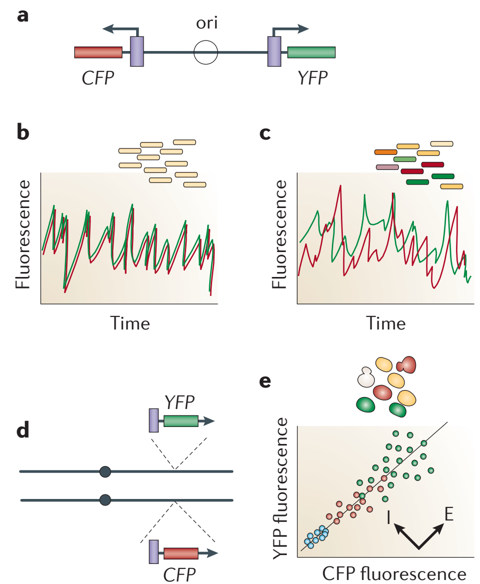
Box 1 | **Analysis of noise in single cells of *Escherichia coli* and *Saccharomyces cerevisiae*.**



The degree to which the expression level of the same gene varies in different cells is an important factor that influences the phenotype of cell populations. For any gene, noise is a measure of this variation and is usually defined as the standard deviation divided by the mean of the distribution of protein-expression levels[11,13].

Measuring noise in prokaryotes

In a prototype experiment that was designed to measure noise in *Escherichia coli*, two copies of a specific promoter — for example, that of the transcriptional repressor of lactose catabolism (*lacI*) gene or of the early promoters of phage λ (shown as blue bars in panel **a**) — are fused to different variants of *GFP* — either *CFP* or *YFP* (represented by red and green rectangles in the figure, respectively) — and placed as single copies at two different locations in the genome[13]. These locations are chosen equidistant from the origin of replication (ori) to minimize variation that is due to chromosomal location. The bacteria are allowed to grow, and read-outs of fluorescence are taken for individual cells over time. If cells make the same amount of each protein they will appear yellow, whereas green or red bacteria indicate increased amounts of either YFP or CFP, respectively. Therefore, the degree of green and red is a measure of the variation (noise) of the population.

In the absence of intrinsic noise (panel **b**), there should be no fluctuation in the relative amount of protein at the single-cell level: the expression of the two proteins will correlate over time and all cells will be yellow. Cells will be yellow in synchrony, even though the amount of protein varies between cells owing to extrinsic noise. If there is intrinsic noise (panel **c**), cells will be different colours and there will be no correlation between the expression of *CFP* and *YFP*. Fluorescence is a measure of protein concentration and therefore also of gene expression.

Measuring noise in eukaryotes

A similar experiment can be carried out in eukaryotes, where, owing to diploidy, two genes are present in homologous locations in the same cell (panel **d**). As with bacteria, it is possible to label one allele with *YFP* and the other with *CFP*, and to monitor the expression of these reporters[12]. Noise can then be quantified by plotting measurements of fluorescence from the two genes in individual cells (panel **e**). In the figure, different coloured circles represent cells that have been sampled from a culture at different time points. Any spread that is perpendicular to the diagonal (where the intensities of the two genes are equal) reflects intrinsic noise (I). A spread that is parallel to this line represents extrinsic noise (E). This basic technology can be used in various experimental set-ups to monitor the performance of genes and gene-regulatory networks.

at the crucial values of the transfer function. This sensitivity of the system to the spread of noise can lead to highly variable outputs in a cell population, which can be exploited by natural selection.

Real GRNs can have intricate designs (see, for example, the specification of endomesoderm in the sea urchin[22]) and are therefore sensitive to the spread of noise. How then is this potential for random behaviour curbed? In bacteria and yeast, one element of control lies in the deployment of network motifs[23–26] (FIG. 2a–c). These motifs function as information-processing devices and reduce noise, and thereby make the information processing more effective; the architecture of the network therefore influences its performance[26–28]. Under certain circumstances these motifs not only filter out noise but also create sensitivity to inputs, and endow the system with special behaviours, most notably bistability[29,30] (FIG. 2d,e). Bistability confers robustness to the system because the switch from one state to another is not easily reversible, and single cells in a system can explore the possibilities of a new parameter space unhindered[29,30]. The behaviour of the *lac* operon of *Escherichia coli* under inductive conditions[29] or the lysis–lysogeny decision in phage λ[31] are simple examples of bistability at the transcriptional level.

These studies have revealed that the performance of a GRN is intimately linked to its architecture and to small changes in some variables[27,32]. However, these conclusions are based on engineered experiments in which variables are carefully controlled and measured.

Developmental networks. The architecture of developmental GRNs is beginning to be unravelled[22,33,34]. In contrast to the thin-layered regulatory architecture of *E. coli*[26], eukaryotic developmental systems have a dense, deep and capriciously complex structure[34,35]. They are branched and their transition points often involve cohorts of 20 to 100 genes, the expression of which has to be coordinated over cell populations[36,37]. Simple extrapolation of what has been gauged from bacterial systems indicates a potential for the spread and amplification of noise in developmental GRNs. However, as their performance is reliable and reproducible there must be extrinsic controls over the spread of noise, not to mention fine-tuning of the system.

Variability of gene-expression levels in a population of bacteria or yeast can be advantageous, as it provides

Endomesoderm
The group of cells that give rise to the endoderm and mesoderm.

Network motifs
Stereotyped patterns of interactions between the basic regulatory elements of a network in bacteria and yeast. They are defined as the functional interactions between the elements of a network that occur in real networks more than other possible interactions.

Bistability
The observation that for a certain range of parameters the system can exist in either of two (or many — multistability) stable states.

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a substrate for selection. However, this property is not desirable for a developmental decision in a multicellular organism during which one, or more often many, cells have to express many genes in a stable manner and over a defined period of time to allow successive patterning events to take place. Bistability is probably an important property of each element of the network, and because the expression of, say, 50 genes might need to be coordinated for a simple fate decision, it is easy to see how any fluctuation or variability in gene expression will be amplified, with detrimental consequences. In particular, noise will promote random combinations of expressed genes in different cells. Development is not simply about turning genes on and off but about doing this at the correct spatio-temporal coordinates, for which definitions are, naturally, also subject to fluctuation and will contribute to noise in the final output. Therefore, the natural tendency of a developmental GRN might be towards randomization and chaos unless there are specific regulatory mechanisms to curb this.

The buffering of noise in a developmental process has been shown during the segmentation of the early embryo of *Drosophila melanogaster*. At the top of the segmentation-gene hierarchy is the gradient and activity of Bicoid, a transcription factor that functions as a concentration-dependent switch for particular genes, and ultimately leads to a fine-grained pattern of stripes of gene expression along the anterior–posterior (A–P) axis of the embryo[38–40]. Quantitative studies reveal that, whereas the local concentration of Bicoid at a defined point on the A–P axis can vary greatly from one embryo to another, the spatial definition of the expression of its immediate target, *hunchback* (*hb*), does not[41] — that is, the expression of *hb* filters the noise of the Bicoid gradient (FIG. 3). This filtering process reaches the next tier of the regulatory hierarchy, the pair-rule genes[42,43], and therefore indicates the existence of mechanisms that smoothen fluctuations in Bicoid concentration to produce a robust output. Even in this apparently simple case the nature of the mechanism remains unknown. So far, the search for dampening devices has been restricted to the search for mutants that have altered levels of noise[41], but success has been limited.

So, noise is an inevitable consequence of the molecular nature of the process of gene expression and there must be devices in place to deal with it. However, empirical studies and modelling in engineered systems already indicate that the architecture of GRNs might not be enough to curb the potential for variation and noise[19].

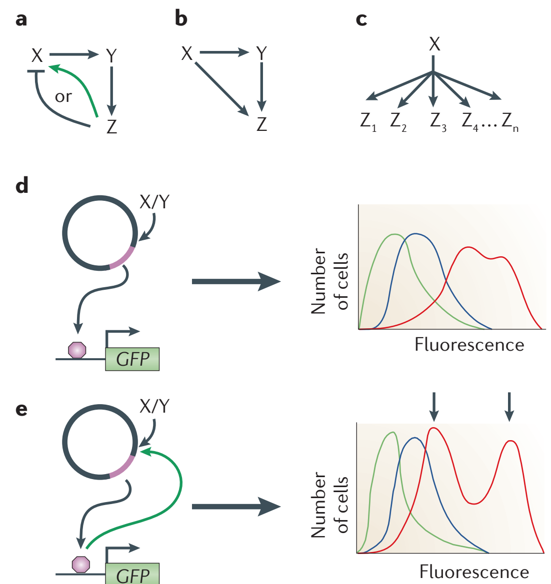

Figure 2 | **Examples of simple gene-regulatory networks and their information-processing capacity.** Work in *Escherichia coli* and *Saccharomyces cerevisiae* has revealed the existence of network motifs[23–25]. These motifs have significant information-processing ability. **a–c** | Classic and well-understood examples of information-processing devices: feedback loop (**a**), feed-forward loop (FFL) (**b**) and single-input module (SIM) (**c**). In this representation of the FFL, expression of Z requires both X and Y, and the network has two significant properties: it only responds to persistent X activity and it rejects rapid variations in X. In the SIM case, the Z_{1-n} genes that are shown could have different thresholds and would turn on and off from low to high threshold (for details, see REF. 28). **d,e** | The architecture of a network is an important determinant of the performance of the system and has an effect on noise. For example, in a simple activation system (**d**), the activity of the transcriptional activator (X/Y) is related in a simple manner to its amount, and the greater the amount of regulator (input), the greater the expression of the regulated gene (output, *GFP* expression). This is shown on the right-hand side at low (green), intermediate (blue) and high (red) levels of inducer. However, inclusion of a positive feedback loop (**e**) changes, at appropriate levels of activity, a continuous response system (**d**) into a bistable one (**e**). At high concentrations of the inducer, the positive-feedback loop leads to a bistable response of the system, with cells having either a low or a high output (arrows in **e**).

Regulating noise during cell-fate assignation

A framework for the analysis of 'cell-fate transitions'. It is not difficult to gauge from the discussion above that there are many sources of noise in eukaryotic gene expression and that there will therefore be several mechanisms to regulate it. Here we focus the discussion of noise in developmental systems on the transitions that occur during cell-fate assignations (FIG. 1). This is because we perceive this process to be a basic unit of information processing that is repeated many times during development, and also because it can be reduced to simple terms, simplifying its description and analysis (FIG. 1).

The object of a cell-fate transition is to establish a stable pattern of gene expression that defines the identity of a cell. We suggest that a transition between two cell states, S1 and S2, can be divided into two steps: induction and stabilization[44] (FIG. 4). The induction phase corresponds to the onset of a gene-expression profile that defines S2. It leads to a probability of expressing a set of genes, but it is not sufficient to establish a stable S2 profile. Induction triggers fluctuating and heterogeneous levels of expression of the S2 genes within a cell and within a cell population (FIG. 4b). A second step is required to

Induction
The definition of a particular and molecularly defined state through an input.

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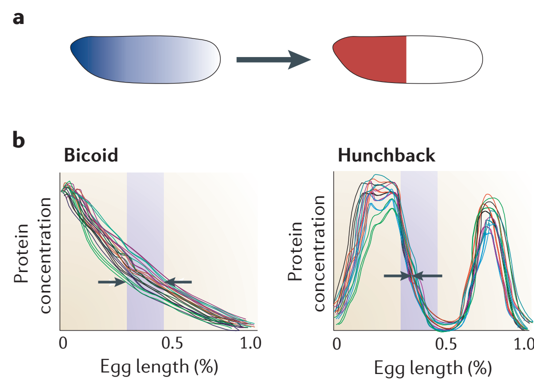

Figure 3 | **Bicoid activity in the early *Drosophila melanogaster* embryo. a** | In the early *Drosophila melanogaster* embryo a gradient of the homeobox-containing protein Bicoid induces gene expression in a concentration-dependent manner. The *hunchback* gene is a direct target of Bicoid. A *D. melanogaster* embryo in the early syncitial stages is shown, with the distribution of the Bicoid protein in a gradient from anterior to posterior (blue) and the distribution of the Hunchback protein at a similar stage (red). **b** | A quantitative examination of the gradient of Bicoid in different embryos shows that there is significant variability in the expression of the protein — that is, the gradient of Bicoid is noisy[41]. The noise is shown at the threshold of target-gene activation (blue band). Surprisingly, however, the response, measured as the expression of *hunchback*, is very precise (compare arrows on left and right diagrams). This means that a noisy input is filtered by the system to produce a precise output. Protein concentration is measured indirectly through fluorescence of antibody-stained embryos. Adapted, with permission, from REF. 44 *Nature Reviews Molecular Cell Biology* © (2003) Macmillan Magazines Ltd.

stabilize expression and therefore establish the S2 state (FIG. 4a,b). This stabilization can be construed as the filtering of noise within a cell and has to be applied to every gene in every cell of a population. In more mechanistic terms we propose that the induction phase corresponds with the opening of the chromatin, the assembly of the transcription-initiation complex and it becoming poised for a particular rate of transcription. By contrast, the stabilization phase is the assembly of a chromatin structure that allows the inductive arrangement to be functional over time and stable against fluctuations.

Theoretical[12] and empirical[45] considerations favour this proposed division of the establishment of gene expression during a cell-fate transition. In eukaryotes, the opening of the chromatin, which generates transcriptionally competent DNA, is a prerequisite for the onset of transcription. In yeast, the activation of the DNA is an infrequent event relative to the actual transcription process[12] and is the rate-limiting step. We propose that this is probably also the case with higher eukaryotes. Here the activation phase corresponds to the S1 to S1/S2 transition, whereas the stable transcriptional state is associated with S2; that is, the S1/S2 to S2 transition is the step and we suggest that, unless it is actively implemented, the cell will always relapse into S1 or some other available stable state.

Syncitium
A multinucleate cell in which the nuclei are not separated by cell membranes.

Lateral inhibition
A developmental strategy that curtails the development of a particular fate in cells that surround a cell that has already adopted this fate.

Community effect
A developmental strategy that results in the development of homogeneous and stable expression levels of a particular gene or genes across a population that initially had low and variable expression levels of those genes.

Strategies to use and dampen noise in development. The idea that a process of cell-fate assignation can be divided into induction and stabilization is supported by the observation that the potency of a cell during development is greater than its actual fate — that is, for a certain amount of time the fate of a cell is not fixed[1–3] (FIG. 4c). In many instances, each cell within a population can adopt a particular fate by virtue of its history and position in the embryo, but only some cells adopt each fate stably. Experimental manipulations have shown that the initial broad distribution of the potential to adopt a fate is a regulative device and provides a basis for pattern formation. In terms of the framework outlined above, for a fate transition from S1 to S2, many cells progress to the S1/S2 state but only some stabilize the S2 state.

The singling out of precursors for the nervous system in *D. melanogaster* through lateral inhibition has provided a model for this developmental strategy. During this process, cells decide between an epidermal (S1) and a neural (S2) fate. Proneural genes shunt cells into an S1/S2 state that is maintained in the population until one cell is tipped towards S2 and uses cell–cell interactions to suppress the others from adopting the same fate. These other cells revert to S1 and either wait for another opportunity to become S2 cells or to adopt another fate (S3 or S4) (FIG. 4c). Little is known about the details of the molecular process that mediates this transition, although Notch signalling is clearly involved in the suppression of the neural fate[46].

Another example of a strategy at the population-level, but one that includes rather than excludes cells from adopting a fate, is the community effect. This was originally described in the context of mesoderm specification in amphibian development[47,48]. In this process, a group of cells is induced to adopt a fate by the product of a tissue-specific gene. Initially, too few cells within the population reach the threshold of expression that is sufficient for the new fate to be adopted. A second event is necessary to raise the global levels of expression, and this is achieved through a positive factor that enhances the community-wide expression of the tissue-specific gene. In contrast to lateral inhibition, which is a fate-suppressing strategy, the community effect is a fate-promoting one. There are currently a few molecular candidates for its implementation[49], but little is known about the details of the mechanism.

During lateral inhibition and the community effect, an initial burst of *de novo* gene expression within a population is followed by the stabilization of this pattern of gene activity in some cells. The initial burst of expression leads to an unstable state, and in both cases transcriptional noise is exploited to ensure that cells have developmental plasticity and that the adoption of a stable fate is a regulated process. Although the architecture of GRNs can contribute to regulating, dampening and filtering noise[26,50], and models exist that show, on the basis of existing data, the robustness of these strategies[35,50–52], it is also possible that specific molecular devices are dedicated to this function. We suggest that a candidate mechanism in developmental systems that is dedicated to the control of noise at the level of gene expression must fulfil a number of properties.

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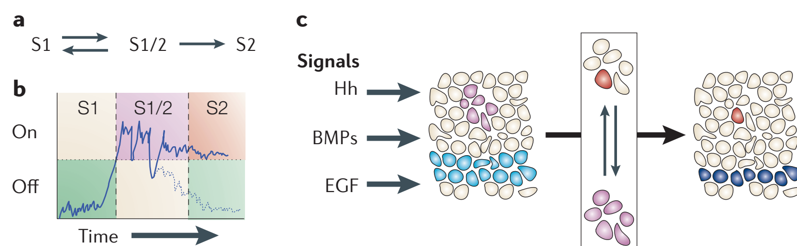

Figure 4 | **A model for the analysis of cell-fate transitions. a** | Transition between two states, S1 and S2, each associated with the expression of many state-specific genes, is a characteristic of cell-fate assignations. The transition can be divided into two phases. Step 1 is the onset of the S2 gene-expression profile in the background of S1, which leads to an unstable and reversible state. Step 2 is the stabilization of the S2 profile, which, in normal development, is irreversible. **b** | Gene expression is associated with noise. This cartoon represents the dynamics of this variable for a particular gene during the S1 to S2 transition. In this example, the S2 state is associated with the stable expression of this gene ('On'), which is noisy. Under normal conditions the expression is stabilized and the S2 state is reached. In the absence of a stabilizing influence, expression decays ('Off') and the cell retains its initial state (dashed line). We propose that in many instances the second step is mediated by Wnt signalling. **c** | Example of a cell-fate transition. In the first step, signalling molecules, in collaboration with the state of a cell, induce new states in several cells that have specific expression patterns (blue and pink). In a second step only some of the cells that initiate the transition come to express the genes stably (dark blue and red), whereas the others lose the ability to adopt that fate. BMP, bone morphogenetic protein; EGF, epidermal growth factor; Hh, Hedgehog.

First, it should function across cellular fields — that is, an ideal noise-filtering system should be a cell-signalling device that can function over a cellular field, as this will allow it to coordinate its effects over large populations of cells. Second, it must target chromatin structure because this is the focus of much of the activity that is important for development (although we acknowledge that chromatin remodelling is only one process in which noise can occur and be regulated). Third, in principle, the noise-filtering mechanism should not be part of the induction step.

In the rest of the article we consider some observations that indicate that Wnt signalling fulfils these requirements.

The function of Wnt signalling

Wnt proteins are a family of extracellular signalling molecules that mediate cell interactions during development[53]. There are two major branches of Wnt signalling: one, which is β-catenin-dependent, targets transcription through a nuclear pool of β-catenin[54]; the second planar-cell-polarity (PCP) branch targets the activity of the cytoskeleton[55]. A third branch is thought to target calcium and protein kinase C and is less well characterized[56].

Wnt/β-catenin signalling and transcriptional noise. Wnt/β-catenin signalling is usually perceived as a module that contributes one more element, often in a concentration-dependent manner, to the combination of factors that pattern tissues during development[8,53] — that is, Wnt has a role in the inductive phase of the S1 to S2 transition (FIG. 4). However, close analysis of the consequences of gain and loss of Wnt signalling (BOX 2) suggests a different view, namely that Wnt/β-catenin signalling does not affect the initiation of target-gene expression but rather its stabilization and maintenance[44,57]. In the context of the two-step model of cell-fate assignation proposed above, Wnt/β-catenin signalling mediates the S1/S2 to S2 transition.

An important premise of our hypothesis is that the Wnt effector, β-catenin, carries out a qualitatively different function on gene expression from that of the effectors of other signalling pathways. Whereas, say, epidermal-growth-factor (EGF), Hedgehog or bone-morphogenetic-protein (BMP) signalling target the transcriptional machinery and establish rates of transcription, Wnt signalling preferentially targets chromatin remodelling and the stabilization of the expression rates and patterns that are set by transcription factors (BOXES 2,3). There is some evidence that this might be the case (see BOX 3 for details). For example, T-cell factor (TCF), the DNA-binding partner of β-catenin, is a high-mobility-group (HMG)-box-containing protein that can activate transcription but that, in general, bends DNA. Furthermore, both TCF and β-catenin interact functionally and physically with elements of chromatin-remodelling complexes and other related proteins[58] (BOX 3).

Studies of transcription at the single-cell level indicate that the function of certain enhancers is not to determine the net rate of transcription, but rather the probability that a gene is on or off in a particular cell[59,60] — in other words, a transition between two states of a bistable system. With this in mind, a corollary of our proposal for the role of Wnt signalling is that certain genes have two classes of signal-responsive elements: those that are associated with setting the rate of transcription, which respond to the conventional signals[61], and those that are associated with the stabilization of the set rate (BOX 3). The transition between S1/S2 and S2 requires the coordination of the two processes. We suggest that Wnt affects the second process and that Wnt-response elements increase the probability that a certain rate is stably established in the cell.

This proposal raises the issue of which genes are targets of Wnt signalling: do all genes need to undergo an S1/S2 to S2 transition? In our proposal, Wnt signalling provides a kind of memory that allows a gene to be stably expressed, and we surmise that there are three classes of gene. Class I genes do not require 'memory' — they are either always on or are quickly turned on and off, and do not require chromatin remodelling or stable chromatin structures (BOX 3a). Examples include genes that encode components of metabolic networks and rapidly inducible genes, such as those that are involved in the early phases of wound healing. Class II genes are at the opposite end of the spectrum and require 'long-term memory' — their transcriptional state has to be maintained for days or years. The Hox genes, for which long-term memory is mediated by members of the Polycomb and Trithorax gene classes, are an example of this class. Class III consists of genes that require 'short-term memory' — they need to have stable chromatin structures for just long enough to maintain a particular state, but not for years (BOX 3b,c). Some class III genes might also need 'long-term memory'. We suggest that the memory of Class III genes, which is important for developmental purposes, is provided by Wnt signalling.

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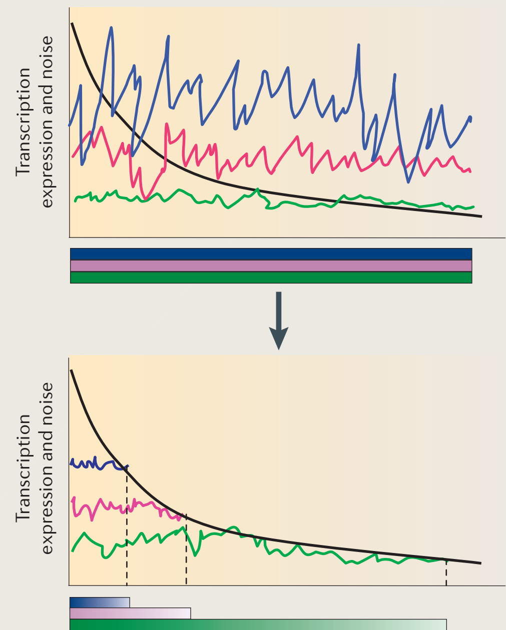
Box 2 | Effects of gain and loss of function of Wnt signalling

The contention that Wnt signalling can filter fluctuations in gene expression and influence the probability that a cell adopts a fate, rather than influencing the fate itself, is supported by the analysis of loss and gain of function of Wnt/β-catenin signalling.



Loss of function

In the absence of Wnt, cells undergoing an S1 to S2 transition begin to transcribe genes that are associated with S2, but the 'noisy' S1/S2 state never resolves into a stable S2 state (FIG. 4a,b). The three examples that are described below demonstrate this point.

Early in fly development the expression of *engrailed* requires Wingless for its stable expression[75,76]. In the absence of *wingless*, *engrailed* expression is initiated but subsequently decays in a random manner[77]. A similar response to the loss of *wingless* signalling is observed in the patterning of the PNS of the adult fly: a Wingless-responsive enhancer of the Achaete–Scute complex drives expression of *achaete* and *scute* in precise positions in the adult epidermis. In *wingless* mutants, the activity of the enhancer is not abolished, but rather becomes reduced and variable from individual to individual[78]. Similarly, in early embryos of mice that are mutant for *Wnt3a*, the expression of *brachyury*, a Wnt target, is initiated correctly but decays without peaking[79].

Gain of function

Ectopic activation of Wnt signalling rarely, if ever, triggers ectopic or *de novo* activation of gene expression; instead, it homogenizes patterns of gene expression over pre-existing competence domains or simply increases the levels of already established patterns of gene expression[78,80–88] (reviewed in REFS 44,57). A recent analysis of the role of Wnt signalling on the activation of vertebrate epidermal stem cells[86] supports many of our conclusions — hyperactivation of Wnt signalling during the activation of these stem cells in the mouse shows that the main function of Wnt signalling is to coordinate and lower the response threshold of these cells to other signals that activate them, rather than to specify particular fates[86].

The results of these studies are consistent with the notion that Wnt signalling affects the stabilization rather than the initiation of gene expression[44,57].

In some cases, gradients of Wnt signalling are created from fixed spatial sources of Wnt protein expression (see figure). A graded concentration of a Wnt protein (black line) that functions on genes that have similar domains of potential expression but different levels of expression and intrinsic noise (coloured traces) can create nested responses of stabilized patterns of gene expression that mimic the activity of morphogen gradients. In this case, three genes come to be expressed over a similar domain but have different levels of expression. The differential responses to Wnt are likely to reflect the sensitivity of the responding genes to the filtering effects of Wnt, rather than the concentration-dependent onset of gene expression.

Competence domain
A group of cells that share the potential to adopt a particular fate.

Transdetermination
The property of cells to spontaneously change their commitment from one particular developmental trajectory to another.

Imaginal disc
A specialized groups of cells in Diptera that give rise to adult structures such as wings or eyes. They are set aside during embryogenesis, proliferate inside the larva and then produce the adult structures during pupation.

Our proposal provides a context in which to interpret some of the existing observations about Wnt signalling, and might help to explain certain features about the role of this pathway in fly transdetermination and mammalian oncogenesis. In transdetermination, cells from a particular imaginal disc change their state of determination under the influence of Wingless[62]. It might be that high levels of Wingless stabilize the fluctuating patterns of gene expression that are present in the wild type and would otherwise not be stabilized. A similar situation might apply to the role of Wnt/β-catenin signalling in tumorigenesis[54,63]. In certain cell types the inappropriate activation of Wnt signalling might lead to the amplification and stabilization of low fluctuating levels of potential oncogenes. This effect might be particularly significant in situations in which Wnt signalling is normally off and its threshold of activation is low — for example, at the boundary in the intestine between proliferating and differentiating cells, where activation of Wnt signalling easily leads to tumour formation[54,63].

Wnt/β-catenin signalling as a noise filter in development? Wnt signalling seems to fulfil the three conditions that we set above for a molecular filter that is dedicated to the control of noise during gene expression in development: Wnt proteins can spread over large cell populations and therefore have long and wide ranges of activity; β-catenin, the transcriptional effector of the pathway, shows extensive interactions with the chromatin-remodelling machinery; and the signalling events are not involved in the initiation of transcription, but rather in its maintenance.

In some cases Wnt signalling seems to function in the inducing phase, but a careful analysis leads to a

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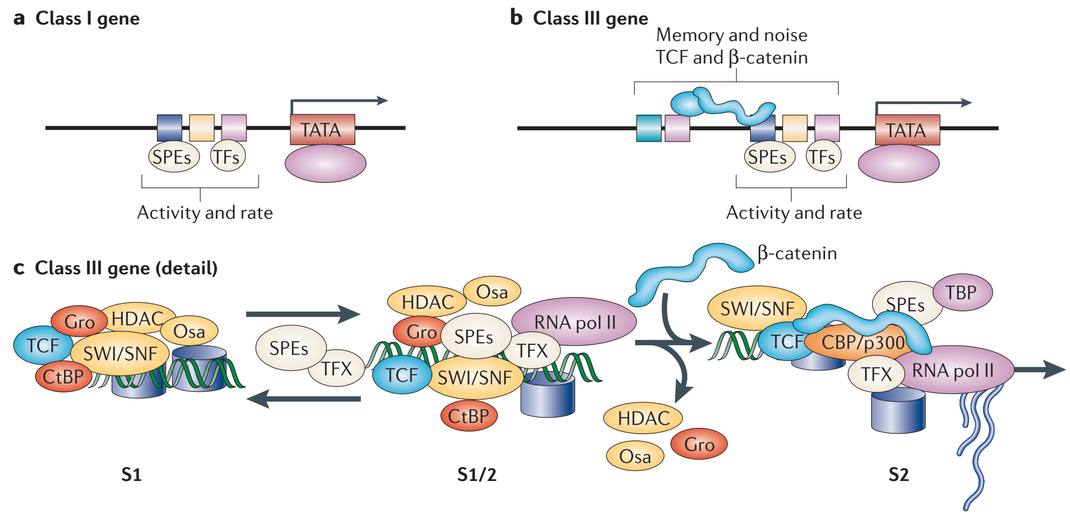
Box 3 | **Wnt signalling — a filter for transcriptional noise?**



Wnt signalling fulfils some of the criteria for a filter of transcriptional noise. The evidence for such a role rests on the effects of gain and loss of function of Wnt/β-catenin signalling (BOX 2) and on the nature and number of β-catenin-interacting proteins. There are two such groups of proteins. One comprises the many proteins that are involved in chromatin remodelling (see supplementary information S1 (table)); for example, in *Drosophila melanogaster*, lowering the activity of components of the chromatin-remodelling machinery (such as Osa) does not have a pleiotropic effect but rather results in a Wnt loss-of-function phenotype[89]. This indicates that a large component of the Wnt signal is concerned with imposing a structure on the chromatin of enhancer elements, therefore stabilizing target-gene expression. A second group of β-catenin-interacting proteins are promoter-specific, are shared with the effectors of other signalling pathways — such as Smad, Cubitus interruptus and Notch — and probably define the context in which the chromatin-remodelling machinery operates.

Our proposed model for how Wnt signalling filters noise and dampens fluctuations in gene expression is shown in the figure. A promoter of a class I gene (that is, one that is either always on or is quickly turned on and off) does not need stable chromatin remodelling (panel **a**); its activity is simply dependent on the interaction between the basal transcriptional machinery (pink) at the TATA box, and the transcription factors (TFs) and signalling pathway effectors (SPEs), both of which are shown in beige at the coloured binding sites. These interactions determine the rate and activity of transcription.

A class III gene (panel **b**) requires short-term memory. This is provided by the stable chromatin configuration that is created by the interaction of T-cell factor (TCF) with β-catenin (both shown in blue), which stabilizes the chromatin configurations that are defined by the TFs and SPEs in the manner shown in panel **c**.

In the absence of Wnt signalling, TCF family members bind to specific targets and repress expression by associating with transcriptional repressors such as Groucho (Gro) or C-terminal binding protein (CtBP, shown in red), and by recruiting chromatin-remodelling complexes that include histone acetylases (HDACs, shown in yellow) such as Rpd3. This arrangement probably keeps the chromatin in a 'closed state' at these loci. At defined times and locations, signals and transcription factors (SPEs and TFX, which represents a promoter-specific transcription factor, X) interact at these promoters in the S1 to S1/S2 transition to induce a poised, noisy, unstable state of gene expression, which on its own does not result in a stable transcriptional state. Wnt signalling activates β-catenin in the S1/S2 to S2 transition and brings it to TCF. This results in the displacement of repressors and the recruitment of activators such as Brg-1 (not shown), transcriptional co-activators CBP (CREB-binding protein) and p300 (both shown in orange), as well as components of the basal transcriptional machinery such as TATA-box-binding protein (TBP). As a result the expression of the genes defined in the first step becomes stable and robust.

RNA pol II, RNA polymerase II; SWI/SNF, switching deficient/sucrose non-fermenting.

different interpretation. If, for example, gene X fails to be expressed in the absence of Wnt, this could be due to the lack of Wnt signalling in an earlier event that determines the expression of another gene that is necessary for the induction of X, rather than a direct requirement for Wnt. This situation has been shown recently for the role of Wingless signalling in the relationship between *twist* and *slouch* expression in the somatic mesoderm of *D. melanogaster*[64]. Expression of Twist in the mesoderm precedes the expression of *slouch* in muscle founder cells, and loss of Wingless signalling results in low levels of Twist and no *slouch* expression. However, forced high levels of Twist in the absence of Wingless rescue the loss of *slouch* expression. That is, *slouch* expression requires a certain threshold of Twist activity and, in the wild type, Wingless signalling lowers this threshold to allow normal low levels of Twist to function. High and sustained levels of Twist can bypass the requirements for Wnt, which underlines the role of Wnt in establishing thresholds and filtering noise. There are probably many situations similar to this and we hope that our proposal encourages a more detailed and critical analysis of Wnt-mutant phenotypes.

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Wnt/PCP signalling and cytoskeletal noise. The analysis of molecular noise in biological processes has so far been focused on the gene-expression machinery, but it probably also applies to signalling, the activity of the cytoskeleton and other aspects of cell biology. The activity of the cytoskeleton is of particular interest in this context because, much as the polymerase has to engage stably with DNA and RNA has to engage stably with ribosomes, the organization of microtubules and actin polymers has to overcome random fluctuations in the nucleation steps that lead to their spatial localization. It is interesting that the PCP branch of Wnt signalling has been implicated in regulating the activity of the cytoskeleton and that, as in the case of Wnt/β-catenin signalling, the Wnt/PCP pathway seems to be involved not in establishing patterns of cytoskeletal activity, but in their maintenance or coordination[65–67].

Wnt/Notch signalling and noise

Establishing whether Wnt signalling functions as a general filter for noise will require further experiments; it is too early to say whether there are other signalling systems or devices that are dedicated to the same operation. As indicated above, Notch signalling seems to have an important function in influencing cell-fate assignation in development by suppressing cell fates through a conserved mechanism[46]. This process could be construed as filtering noise. However, there are significant differences between the activity of Notch and that of a noise filter in the sense that has been proposed in this article. Notch signalling suppresses gene expression, which is different from smoothening fluctuations in gene expression towards a state that is either active or inactive. In addition, canonical Notch signalling requires cell–cell contact and therefore cannot operate over several cell diameters, as would be required in a growing cellular field.

Nonetheless, Notch signalling seems to have some role in controlling noise during patterning events. In this regard, it is interesting that Notch and Wnt signalling seem to work together in several developmental events and that they might be functionally linked[68–70] In particular, it has been suggested that Notch is involved in setting up a threshold for Wnt signalling[71]. Such a mechanism would contribute to sharpening the response to Wnt signalling and therefore enable many patterning processes. Furthermore, the two processes might be intricately linked during cell-fate assignations. In the framework that is proposed in FIG. 4, Notch signalling would therefore provide a balancing influence for the action of Wnt.

Conclusions and perspectives

An important requirement of molecular systems that are involved in development is an ability to smoothen out their inherent fluctuations at the cellular level. Understanding how this is achieved is key to understanding why events that are noisy at the mesoscopic level (such as gene expression, signal transduction or cytoskeletal activity) are so reliable at the macroscopic level (cell fate, shape or dynamics). In the near future, and in addition to mapping interactions and outlining the circuitry of developmental GRNs, it will be important to measure parameters of gene expression at the single-cell level and observe how they are modulated at the population level. For example, it will be important to devise single-cell reporter systems, as well as cell-tracking methods that will allow us to carry out high-throughput studies in cell populations *in vivo*. Many of these will be extrapolated from current studies in bacteria and yeast, but we will probably need to develop specific methods for developmental systems.

In the first instance these methods should allow us to identify mechanisms that affect the rate of transcription of a given gene and tell whether the gene is on or off in a single cell, as well as giving accurate measurements of noise in developing cell populations. As we move towards a more quantitative analysis of development, new processes and new perspectives on old processes will emerge. The advances will not just be centred on the analysis of transcriptional processes. Cell signalling is an important component of the cell state and the development of technologies that allow the measurement of integrated responses in multi-parameter spaces within single cells[72–74] will also have a central role in the understanding of how individual behaviours become global ones.

Development is not just about how a collection of equally weighted signalling pathways and transcription factors create spatially ordered cellular diversity through linear programmes of gene expression. It is also about modulating the flow of information, correcting the natural errors that arise from the molecular make-up of the cells, and using and controlling the noise that is inherent in the underlying molecular processes. We suggest that the control of noise during development and pattern formation involves a dedicated molecular machinery, of which Wnt signalling seems to be an important element in multicellular systems.

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Competing interests statement
The authors declare no competing financial interests.

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ARP8 | *brachyury* | *engrailed* | *GCN5* | *hunchback* | *lacI* | *PHO5* | *slouch* | *SNF6* | *twist* | *Wnt3a*
UniProtKB: http://us.expasy.org/uniprot
Achaete | Bicoid | β-catenin | CtBP | Cubitus interruptus | Groucho | Hedgehog | Notch | Osa | Polycomb | Rpd3 | Scute | TBP | Trithorax | Wingless

FURTHER INFORMATION
Alfonso Martinez Arias's homepage:
http://www.gen.cam.ac.uk/Research/martinezarias.htm

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
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