Physics of Living Systems

The zoo of the gene networks capable of pattern formation by extracellular signaling

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eLife Assessment

The study presents **valuable** theoretical insights by attempting to classify pattern-forming gene subnetworks and exploring their potential mechanisms. However, the results are **incomplete**, as they rely on oversimplified models, limited classifications, and assumptions that may not hold in more complex or realistic scenarios.

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Abstract

A fundamental question of developmental biology is pattern formation, or how cells with specific gene expression end up in specific locations in the body to form tissues, organs and, overall, functional anatomy. Pattern formation involves communication through extracellular signals and complex intracellular gene networks integrating these signals to determine cell responses (e.g., further signaling, cell division, cell differentiation, etc.). In this article we ask: 1) Are there any logical or mathematical principles determining which gene network topologies can lead to pattern formation by cell signaling over space in multicellular systems? 2) Can gene network topologies be classified into a small number of classes that entail similar dynamics and pattern transformation capacities?

In this article we combine logical arguments and mathematical proofs to show that, despite the large amount of theoretically possible gene network topologies, all gene network topologies necessary for pattern formation fall into just three fundamental classes and their combinations. We show that gene networks within each class share the same logic on how they lead to pattern formation and hence, lead to similar patterns. We characterize the main features of each class and discuss how they constitute an exhaustive zoo of pattern-forming gene networks. This zoo includes all gene networks that, to our knowledge, are experimentally known to lead to pattern formation as well as other gene networks that have not yet been found experimentally.

Significance Statement

A fundamental question of developmental biology is pattern formation. In this article we ask: 1) Are there any logical or mathematical principles determining which gene network topologies can lead to pattern formation by cell signaling over space in multicellular systems? 2) Can gene network topologies be classified into a small number of classes that entail similar dynamics and pattern transformation capacities? We show that, despite the large amount of theoretically possible gene network topologies, all gene network topologies necessary for pattern formation fall into just three fundamental classes and their combinations. We show that gene networks within each class share the same logic on how they lead to pattern formation and hence, lead to similar patterns.

Introduction

Development is the process by which the intricate complexity of multicellular organisms is constructed from a single fertilized egg or some simple vegetative structure (Fusco and Minelli, 203). Not many other natural processes lead to so much complexity in such a short time. Development entails a natural process of pattern formation: specific cells and cell types end up in specific positions in space (i.e., anatomy). This process of pattern formation can be seen as the generation of spatial information (i.e., information about where each cell and cell type are) from previous information (e.g., information within the fertilized egg). This latter information includes the DNA but also spatial information in the form of compartments with different proteins and RNAs in different spatial locations within the oocyte. In most species, development cannot proceed if this latter spatial information is eliminated experimentally (Gilbert and Barresi, 2023). This initial spatial information within the oocyte arises from spatial asymmetries in the mother's gonads or from the environment (Gilbert and Barresi, 2023). Pattern formation in development, thus, does not usually start from a spatially homogeneous initial condition but from an initial condition that is relatively simple but spatially heterogeneous.

Development can be seen as a sequence of transformations between initial developmental patterns and latter developmental patterns (what we call *resulting patterns*) over developmental time. By developmental pattern, or simply pattern, we mean a specific distributions of cells and gene product concentration over space (**Figure 1**). In most animals, the earliest patterns arise from the division of the fertilized egg into different cells that, thus, inherit different parts of the fertilized egg and different proteins and RNAs (Gilbert and Barresi, 2023). Some of these latter molecules act as transcriptional factors that lead to the expression of further genes, i.e., the transcription and translation of genes into gene products (Gilbert and Barresi, 2023). Some of these gene products are secreted into the extracellular space. In here, we call these molecules extracellular signals but in the literature they are also called morphogens, growth factors, paracrine factors, etc. (Gilbert and Barresi, 2023).

Extracellular signals diffuse in the extracellular space and bind to specific receptors in distant cells (Gilbert and Barresi, 2023). Cells can respond to these signals by changing gene expression and, often, by secreting additional extracellular signals and regulating cell behaviors such as cell division, cell contraction, cell adhesion, cell death, etc. (Salazar-Ciudad *et al.* 2003; Gilbert and Barresi, 2023). The former type of response leads to further changes in gene expression over cells (i.e., further pattern transformations), while the latter leads to cell movement and, consequently, to changes in the distribution of cells and gene expression over space (i.e., further pattern transformations) (Salazar-Ciudad *et al.* 2003).

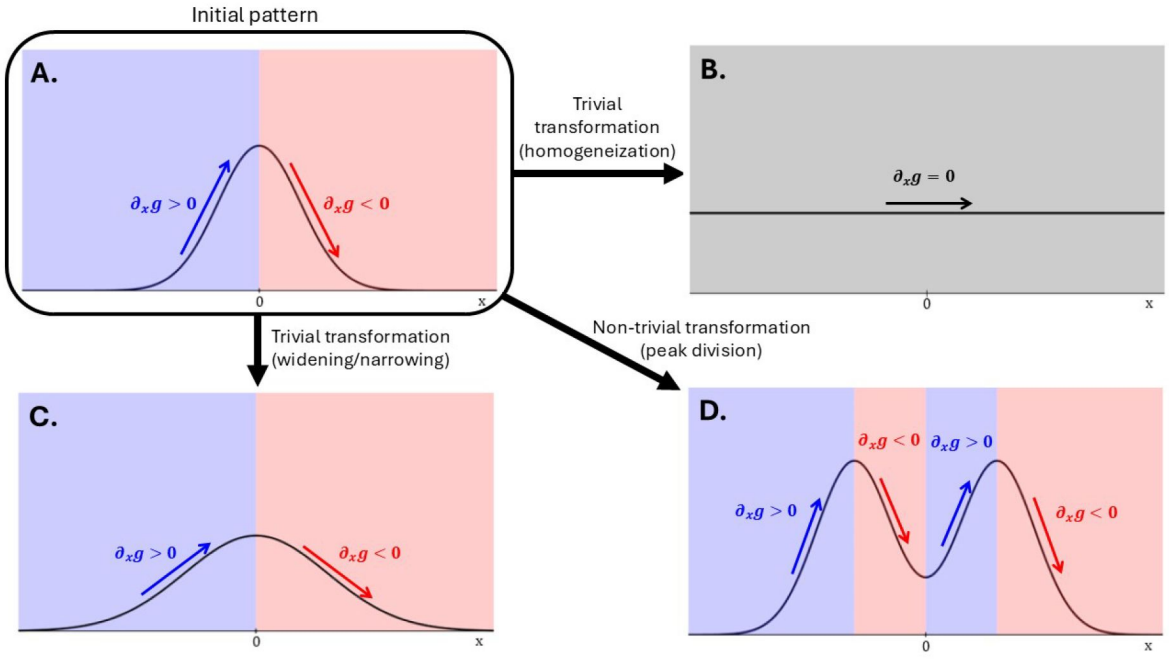


Figure 1

Non-trivial pattern transformations.

The figures shows four patterns (i.e., four spatial distributions of a gene product concentration) an initial one (**A**) and three resulting ones **(B-D)**. The transformation from **(A)** to **(B)** is trivial because the resulting pattern is homogeneous. From **(A)** to **(C)**, the pattern transformation is trivial because the sign of the spatial derivative of the gene product concentration (shown in blue when positive and in red when negative) is the same as in the initial pattern **(A)**. The transformation from **(A)** to **(D)** is non-trivial because the resulting pattern is heterogeneous and the sign of the spatial derivative of$g$ (x) in **(A)** and **(D)** is different (see section S1 in SI for details).

How cells respond to extracellular signals depends on the signal receptors and signal transduction pathways they express (Gilbert and Barresi, 2023). Signal transduction pathways are actually networks of molecular interactions that integrate incoming signals to determine cell responses. In here, we use the term gene network to refer to these networks of interactions, even if they also include molecules that are not gene products (Gilbert and Barresi, 2023).

A fundamental question of developmental biology is how pattern formation, or more precisely pattern transformations (Salazar-Ciudad *et al.* 2003), occur. In this article we restrict ourselves to pattern transformation through gene networks and cell signaling (i.e. no cell division, cell contraction, no extracellular matrix secretion or any cell behaviors leading to cell movement). We ask:

1. Which are the gene networks topologies that can lead to pattern transformation?
2. Can these topologies be classified into a small number of classes with similar topologies and leading to similar pattern transformation?

In this article we only consider non-trivial pattern transformations in which cells do not move. By this we mean pattern transformations in which there are changes on which cells have higher concentrations of some gene product than their immediate neighbors. Non-trivial pattern transformations, thus, imply that at least one gene product exhibits new concentration peaks or changes in the position of concentration peaks (see **Figure 1**). We exclude the cases in which simply, a gene starts to be express in the same pattern than a gene that was already expressed (see section S1 in SI for a more formal consideration). We only consider developmental patterns that are stable in time.

There are several previous theoretical studies exploring how gene products can be wired to lead to pattern transformations through cell signaling (Salazar-Ciudad *et al.*, 2000, 2001; Cotterel and Sharpe, 2010; Marcon *et al.*, 2016; Zheng *et al.*, 2016; Jimenez *et al.*, 2017; Diego *et al.*, 2018; Leyshon *et al.*, 2021). Some of them are restricted to networks of three gene products (Cotterel and Sharpe, 2010; Zheng *et al.*, 2016) while others restrict themselves to study a specific class of gene network topology (Marcon *et al.*, 2016; Zheng *et al.*, 2016; Jimenez *et al.*, 2017; Rand *et al.*, 2021; Leyshon *et al.*, 2021). In a previous study we addressed the same question that in here but through numerical simulation. We found two classes of pattern-formation gene network topologies (Salazar-Ciudad, 2000). This previous study, however, did not show why the identified topology classes are the only possible ones and, due to its purely exploratory approach, it failed to identify one class. In here we take a more general mathematical approach to identify and characterize all possible classes; with any number of gene products.

It is worth noting that there is an abundant theoretical literature on the topic of gene networks in cell biology (e.g., metabolism, gene regulation for cell basic functions, etc.). However, the topic of this article is fundamentally different to those. Here we are not dealing with single cell gene networks, but with systems of cells with identical gene networks that are coupled through extracellular signaling. Specifically, we are interested in those such networks that lead to the phenomenon of pattern transformation (that is an inherently spatial question).

In this study we consider three broad types of initial patterns (i.e., initial conditions): homogeneous initial patterns, spike initial patterns and combined spike-homogeneous initial patterns. In an homogeneous initial pattern, all gene products have the same non-zero concentration everywhere except for some small random fluctuations due to the noise intrinsic to the molecular level. In a spike initial pattern, the concentration of all gene products is zero everywhere except in a cell. At this cell, one or more gene products have a non-zero concentration. As we will see, the secretion of an extracellular signal from this spike leads to an extracellular concentration gradients centered in the spike. The combined spike-homogeneous initial patterns consist of a spike on an otherwise non-zero homogeneous initial pattern (see **Figure 2**). Any

other initial pattern can be constructed by combining spikes of different heights (i.e., different gene product concentrations) at different positions. We then study which gene networks can transform these initial patterns into other (i.e. resulting patterns) non-trivial ones.

Methods: The model

Let us consider a set of N_c cells, each with an identical gene network of N_g gene products. For the purpose of clarity we will explain our results as if these cells have a simple arrangement in space (e.g., a 1D line or a 2D square lattice) but, as we will discuss, our results shall apply with the same logic to any distribution of cells in space. Even though we will only use the term gene product, our model should apply to any kind of biological molecule whose concentration changes as a consequence of the concentration of other molecules. We consider two types of gene products: intracellular gene products and extracellular signals. For intracellular gene products, $g_i(t, x)$ denotes the concentration of gene product i inside the cell in position x at time t >0. For extracellular signals, $g_i(t, x)$ denotes the concentration of the extracellular signal i in the extracellular space surrounding the cell in position x at time t >0.

The dynamics of the gene product concentrations in our model obeys the following system of N_g equations (Murray 2002),

$$\partial_t g_i(x,t) = f_i(g(x,t)) - \mu_i g_i(x,t) + d_i \nabla^2 g_i(x,t), \qquad (1)$$

where $g(x,t) = (g_1(x,t), \ldots, g_{N_g}(x,t)) \in \mathbb{R}^{N_g}_{\geq 0}$ is the vector of concentrations of each gene product at position x; $f = (f_1, \ldots, f_{N_g}) : \mathbb{R}^{N_g} \to \mathbb{R}^{N_g}$ is a function that takes a vector of concentrations as input and returns another vector of concentrations as output; μ_i is the intrinsic degradation rate of gene product i (i.e., the default rate at which a gene product would be degraded by cellular and extracellular proteases if no other factors affect its degradation); and d_i is the diffusion coefficient of gene product i in the extracellular space.

The last term in (1) is Fick's second law (Fick, 19855) describing how the concentration of a molecule changes due to diffusion. ∇^2 denotes the Laplace operator (i.e., $\partial^2_{xx} g_i(x,t)$ in 1D; $(\partial^2_{xx} + \partial^2_{yy}) g_i(x,t)$ in 2D, etc.). Hence, our model only considers extracellular signals that diffuse in the extracellular space (i.e., no membrane-bound signals).

The first two terms in system (1) can be understood as reaction terms. Function f describes how each gene product affects the production of any other gene product within cells. f defines a directed graph via its jacobian matrix J (i.e., the matrix of first derivatives of f with respect to each gene product concentration) (see S2 in SI for further details), where each node stands for a given gene product and each edge describes an interaction between gene products (**Figure 2B**). This directed graph coincides with the gene network and thus, each different J that we consider defines a different gene network.

The parameters of equation (1) and function f are identical in all cells because they are assumed to be encoded in DNA and DNA is the same in all cells of an organism. f does not specify which gene products actually interact in each cell, but which gene products would interact if they happen to be in the same cell at the same time. When and where this would happen depends on the actual dynamics of a gene network on an initial pattern. For the sake of simplicity, we do not explicitly differentiate between transcriptional factors, receptors or other molecules involved in signal transduction: they are just intracellular gene products in a network.

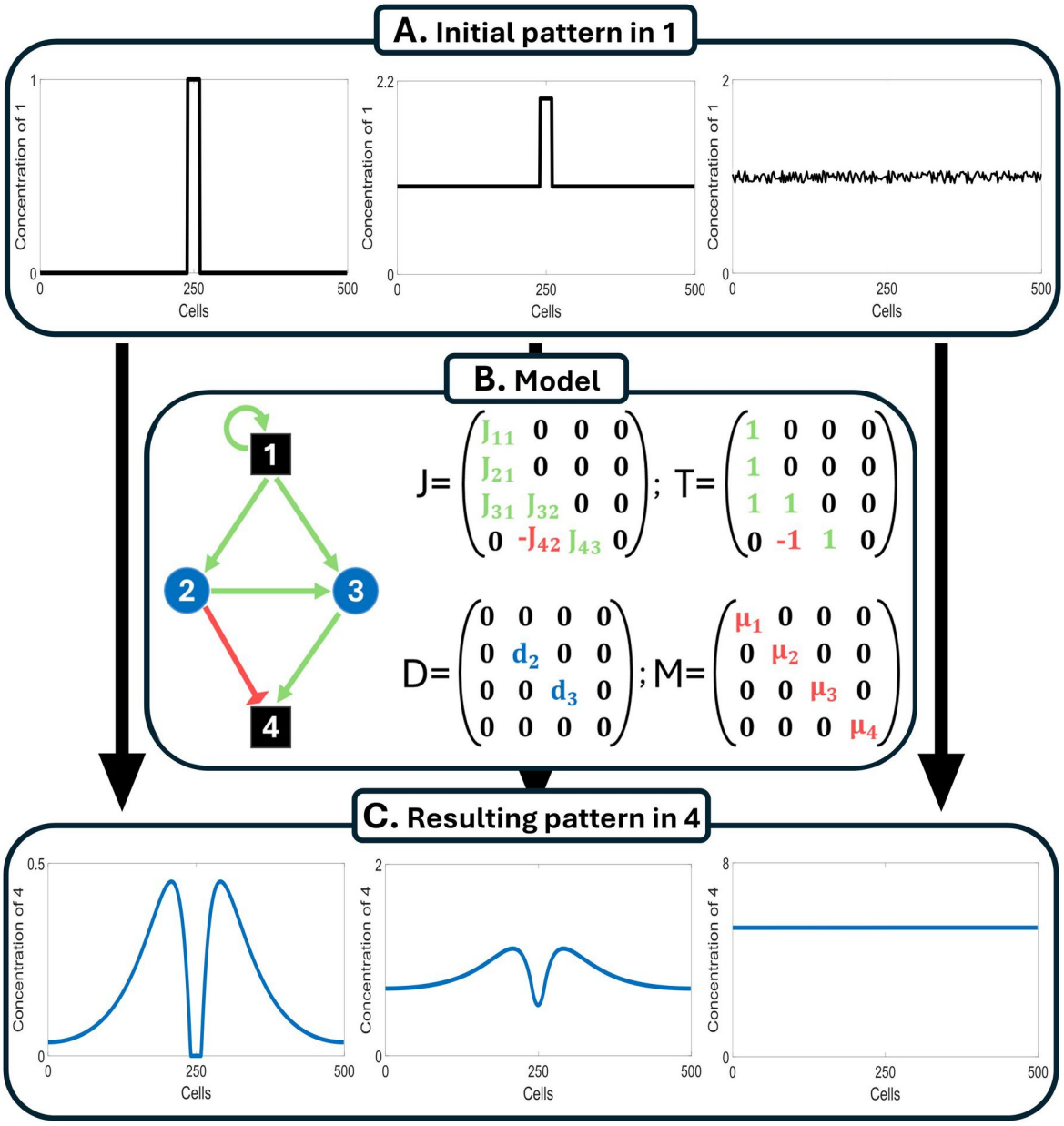


Figure 2

The model transforms initial patterns (A), into resulting patterns (C), through a set of equations implementing gene networks (B).

The article considers three initial patterns **(A)**: spike initial pattern (left), combined spike-homogeneous initial patterns (middle); and homogeneous initial patterns, with small white noise (right). **(B)** Diagram of example gene network. Black squares represent intracellular gene products, blue circles extracellular signals. Green arrows stand for activatory regulations, while red arrows for inhibitory regulations. Weights of the network are given by the*J* matrix; while its topology by the*T* matrix;*D*represents the diffusivities and*M* the degradation rates (see **equation 1**). **(C)** The resulting patterns from each initial pattern in **(A)** under the gene network in **(B)**.

We say that gene product j directly regulates gene product k if the corresponding element of the jacobian matrix is non-zero (i.e., $J_{kj} \neq 0$). This regulation is positive if $J_{kj} > 0$ (i.e., g_j increases with g_k); or negative if $J_{kj} < 0$ (i.e., g_j decreases with g_k). We say j is downstream from k if there exists a chain of regulations between gene products going from k to j. Correspondingly, j is said to be upstream from k (see S3 of the SI for a more formal discussion). Notice that a gene product can be both upstream and downstream of a set of gene products, thus forming a regulatory loop.

In contrast to previous studies (Cotterel and Sharpe, 2010; Marcon *et al*., 2016; Zheng *et al*., 2016; Jimenez *et al*., 2017; Diego *et al*., 2018; Leyshon *et al*., 2021), our results apply to any gene network with any number of genes and any f as long as the latter fulfills a set of very broad biological and mathematical requirements. (R1) f is continuous and continuously derivable (at least locally). (R2) f is non-lineal. This is because a linear f would only lead to zero or infinity concentrations in the long-term (Kondepudi & Prigogine, 2014; Murray, 2002). (R3) f is a function of g_i but not an explicit function of time or any time derivative of g_i. This means that the regulation of a gene product at a given moment depends only on the concentration of other gene products at that given moment, and not explicitly on past concentrations or their rate of change over time. (R4) f is such that g_i is always non-negative and bounded, that is, gene product concentrations are not negative nor infinite. (R5) f is monotonously increasing or decreasing with respect to each gene product concentration. In other words, the partial derivative of f with respect to each gene product, should have the same sign for any value of g_j,

$$J_{kj}(g_1,\ldots,g_{N_g}) = \frac{\partial f_k}{\partial g_j}(g_1,\ldots,g_{N_g}) \geq 0 \ (\text{or} \leq 0), \quad \text{for all} \quad j,k \in \{1,\ldots,N_g\}. \qquad (2)$$

where f_k is the output of f regarding gene product k (i.e., the k-th component of f), and all gene product concentrations, except for g_j, are kept constant. This final requirement simply states that each gene product interaction is always positive or negative (i.e. the sign of the regulation of k by j does not change with the concentration of j). Although, there are known exceptions to this requirement in biological gene networks (Nelson *et al*., 2021), this requirement ensures that complex regulation in our model arises from relatively simple interactions between gene products (i.e., gene network topology) rather than from single gene products depending on complex ways on the concentrations of input gene products (i.e., intricate f_k's). We consider that f can have parameters other than the ones described in (1), as long as these do not change over time and space (i.e., they have to be genetically encoded).

In this article, we assume that our parameter choices for equation (1) and f are such that, in absence of extracellular signals, the intracellular part of gene networks exhibit no complex temporal dynamics such as limit cycles or chaotic behaviors (i.e., their concentrations reach a fixed value). In later sections we discuss some intuitions on how our results may also hold even in the presence of these temporal dynamics.

Finally, we define the topology matrix T of a gene network as the matrix whose elements are − 1, 1 or 0 depending on the sign of the corresponding jacobian element (i.e., $T = sgn(J)$). Studying gene network topology is important because for most developmental systems we only have information about T, but not so much about J (Gilbert and Barresi, 2023). In the results we show that there are only three classes of gene network topologies leading to non-trivial pattern transformation and discuss which types of resulting patterns can arise from each topological class, and their main properties. However, we only provide necessary topological conditions, that is, we show which resulting patterns can be attained from each topological class, but we say nothing about the specific values of μ_i, d_i and J in (1) for which these patterns actually arise. The results are organized into a set of logical arguments (for the least evident of them, we provide mathematical proofs in the supporting information).

Results

At first sight it may seem that the set of possible gene networks is too vast and the possible dynamics of pattern transformation too complex for any meaningful classifications. In this article we will show that this is not the case.

Without cell movement, the only way cells can change their gene expression over space is through cell communication by extracellular signals. No matter how complex is a gene network, cells in one place need to affect cells in other places for pattern transformation to occur and, without cell movement, this requires cell communication. Any network can be partitioned into a set of subnetworks but given that pattern transformation requires cell communication, we focus on partitioning gene networks into one-signal subnetworks. In this article, a one-signal subnetwork is the set of interactions between the gene products downstream of an extracellular signal (and the signal itself). One-signal subnetworks, or from now on simply subnetworks, can partially overlap since a gene can be downstream of several extracellular signals (see **Figure 3**).

Requirements on the intracellular part of gene networks capable of pattern transformation

Here we present two logical requirements that gene network topologies need to fulfill. These are rather trivial but introducing them here facilitates explaining later results.

Requirement I1

For pattern transformation to occur some of the gene products present in the initial pattern must be positively upstream of at least one subnetwork. Without this requirement no signals would be expressed and, thus, no pattern transformations can occur. Similarly, any gene product with a non-zero concentration in the resulting pattern (i.e. the pattern arising from a given pattern transformation) should be positively downstream of at least one gene product present in the initial pattern.

Requirement I2

All gene products are constantly being degraded (second term in (1)). Thus, for a gene product to be present in the resulting pattern it should constantly receive positive regulation from some gene products. This ultimately requires the gene product being within a self-activatory loop or downstream from it (either intracellular or intracellular). This broad case, includes genes that are constitutively expressed, meaning that they are always active regardless of other genes, since this be viewed as these genes being within a self-activatory loop of their own (see section S3 of the SI).

Gene network classification

In this section we explain that all conceivable one-signal subnetworks can be classified into just three classes based on how cells respond to an extracellular signal in terms of secreting, or not, the same extracellular signal (see **Figures 3** and S1). Let Abe the most upstream extracellular signal of a given one-signal subnetwork. Then, there are trivially only two options, either $A$is not downstream of itself (directly or indirectly), or it is. In the former case, we say that the subnetwork is hierarchical, i.e., class H; while in the latter we say that the subnetwork is emergent. The emergent class can be further divided in the L^+class, if$A$is positively downstream of itself, or class L^-, if it is negatively downstream of itself. In other words, in H subnetworks extracellular signals cannot be within any regulatory loop, while in the L classes they are (either with other extracellular signals or with themselves through intracellular gene products).

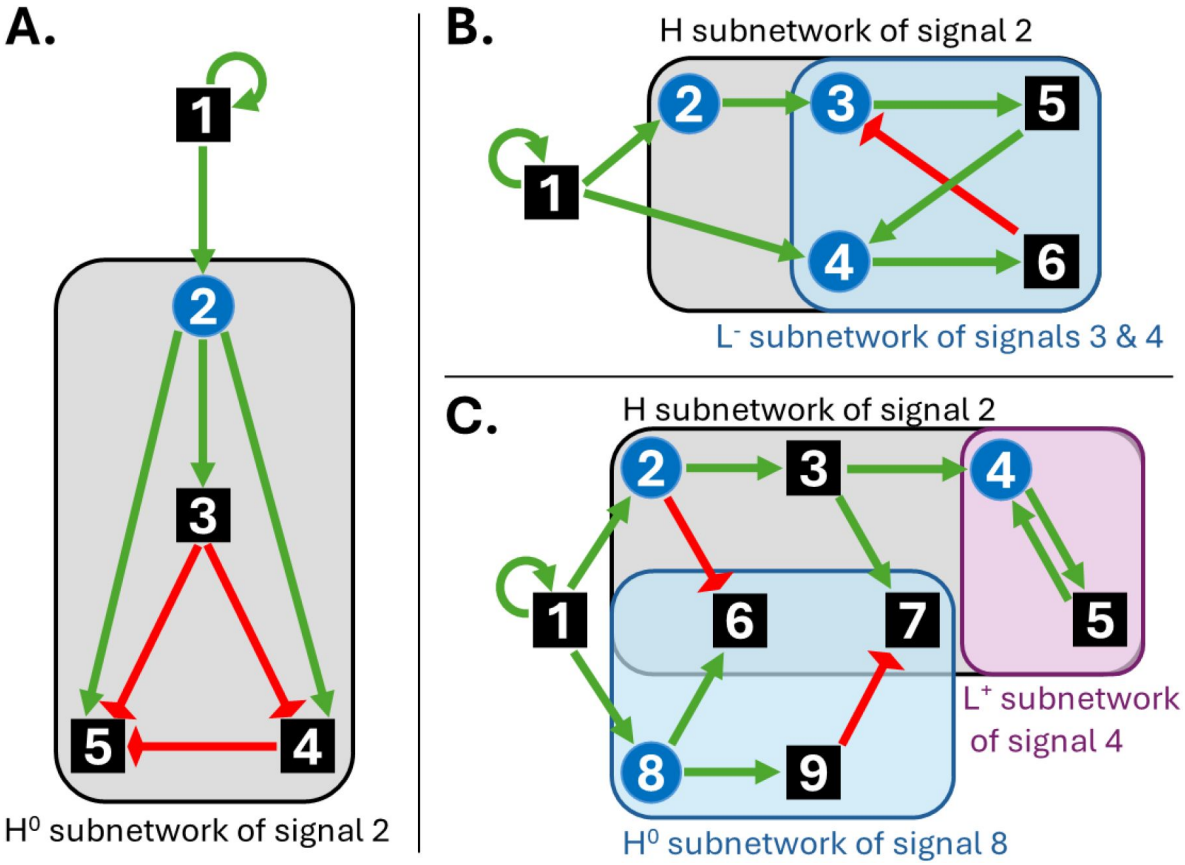


Figure 3

Instances of gene regulatory networks.

The one-signal subnetworks corresponding to each extracellular signal, or pair of signals, are surrounded by a square with a different color. Some subnetworks are part of bigger subnetworks. Network colors and shapes as in **Figure 2**.

Our classification is purely based on topology but, since pattern transformation requires signaling, it is exhaustive: any gene network leading to pattern transformation must include at least one subnetwork, and all one-signal subnetworks fall within these three classes (or their combinations).

Subnetworks can be combined into larger gene networks. We classify whole gene networks in the same way than their composing subnetworks, e.g., a gene network with only H subnetworks is an H gene network. Composite gene networks are labeled according to their composing subnetworks, independently of the number of subnetworks of each class, e.g., a gene network with H and L^+ subnetworks is an HL^+ gene network. In the following sections we will explain which classes of gene networks (i.e., combinations of subnetworks) can lead to non-trivial pattern transformations and which ones cannot.

Hierarchical gene networks

Spike initial patterns: Gradient formation in H gene networks

Let us first consider H gene networks consisting of a single H subnetwork with a single extracellular signal. We call these H^0 networks. From the spike initial pattern, this means that only one cell is secreting extracellular signals (i.e., the one in the spike). Thus, the concentration of extracellular signals in all other cells is fully governed by signal diffusion from this source and its degradation. Indeed, if we consider the set of cells either on the left- or right-hand side of the spike, we can solve equation (1) (see section S5.1 of SI) to show that signal concentrations form an exponential gradient around the spike (see Figure S3),

$$g_A(x)=C e^{-\sqrt{\frac{\mu_A}{d_A}}x}, \text{ for some constant } C>0. \quad (3)$$

Each cell at each side of the spike is at a unique distance to the spike and, thus, experiences a unique concentration of *A* (even if this concentration may barely differ between nearby cells). In that sense, there is a unique relationship between the concentration of *A*and the distance to the spike. This correspondence has been used to propose that with a single extracellular signal, any pattern transformation is possible. The only thing that would be required is that cells interpret signal's concentration in a differentc way for each different resulting pattern attained (Wolpert, 1968, Wolpert, 2016). However, what would that interpretation be, or which would be its underlying gene networks, has not been specified (Horder, 2001). Allegedly, any interpretation should rely on specific gene networks with reasonably simple regulations between gene products, as in our 5 requirement on *f*. A common idea compatible with this approach would be that cells express specific genes if they receive *A*at concentrations beyond some threshold value that is different for different genes. Then different genes may become expressed at different distances to the spike and, thus, a non-trivial pattern transformation would arise (Capek and Müller, 2019; Sharpe, 2019).

There are, however, many other ways in which H^0 can operate. In the following we derive the minimal general requirements that H^0 networks should fulfill to lead to non-trivial pattern transformations (see section S5.2 of the SI for details). The first requirement (RH1) is that at least one gene product positively downstream of *A*, say*k*, inhibits another gene product that is also positively downstream of *A*, say *j*. In other words, there needs to be some inhibition between gene products and, due to requirement I1, these gene products need to be downstream from *A*. This requirement is discussed in previous work by other authors (Monteanu *et al*., 2014), specially for networks with three gene products, but we include it and expanded in here for completeness.

Let us investigate why (RH1) is necessary. If *k*does not inhibit *j*(i.e., if RH1 does not hold), but *A*positively regulates both*k*and *j*, then, according to requirement (R5) on*f*, both*k*and *j* will increase their concentration when *A*does and *vice versa*. Over space, this means that *j*and*k* would exhibit peaks and valleys of concentration in the same places than *A*, and thus, no non-trivial pattern

transformation can occur. Consequently, any gene product exhibiting a non-trivial pattern transformation has to receive a negative regulation and, since in H^0 networks there is only one signal, this inhibition should come, directly or indirectly, from *A*. This fact, together with requirement I1, explains (RH1) (see section S5.2 of SI for details).

For non-trivial pattern formation in H^0 networks, it is also required that either the activation of *k* is non-linearly different to the activation of *j* for different concentrations of *A (RH2a)* or that the inhibition of *j* by *k* is non-linearly higher for high concentrations of *k* than for low concentrations of *k* (RH2b) (see Figure S3 and section S5.2 of SI for further details). Let us first explain RH2a (see Monteanu *et al*., 2014 for a similar discussion). Imagine an H^0 network fulfilling (RH1), but neither RH2a nor RH2b. In that network, *A* regulates *k* and *j* in the same way everywhere and, thus, their concentration over space varies in the same way as that of *A*. This implies that the proportions g_k / g_A and g_j / g_A are the same all over space and, hence, *j* and *k* will have their peaks of concentration in the same place than *A* (i.e., no non-trivial pattern transformation is possible). On the contrary, if the network satisfies (RH2a), then the inhibition of *k* on *j* is stronger close to the spike (where g_k is higher because *A* is higher) than far away from it (where g_k is lower because *A* is lower). This way, a valley of *j* can form close to the spike and, consequently, two peaks of *j* would form at each side of it (Figure S3). See section 5.2 of the SI for why these interactions have to be non-linear. The same changes in the proportions g_k / g_A and g_j / g_A over space can be attained if the network fulfills requirement (RH2b) (see section S5.2 of SI for formal proofs). Notice that in 2D these networks do not lead to peaks but to rings.

Pattern transformations in H gene networks with several extracellular signals

We call H gene networks to the hierarchical gene networks using more than one extracellular signal. These can also lead to non-trivial pattern transformations (Figure S3). In this case inhibition does not need to occur between at least two genes products downstream of A but can occur between gene products downstream from different extracellular signals secreted from the spike. Then, neither requirement (RH2a) nor (RH2b) are necessary if these different extracellular signals have different d, μ or secretion rates (RH2c). In this latter cases, the different extracellular signals only need to attain different proportions between their concentrations over space (e.g. different decay exponents with distance to the spike due to, for example, different diffusion coefficients; see section S5.3 of SI). If this occurs, then inevitably, some of the extracellular signals will have a higher proportion of their concentration close to the spike than others. As in the previous case then, concentration valleys can form around the spike for those gene products that are inhibited by one signal and activated by the other, directly or indirectly (see Figure S3 and section S5.3 of SI for details).

Pattern transformations in gene products further downstream of extracellular signals

Gene products that acquire a pattern through a H (or H^0) network can lead to further pattern transformations in downstream gene products without the need for extra extracellular signals. This would occur in those gene networks in which gene products with a pattern activate gene products that are also inhibited by gene products with a different pattern (Figure S4). The pattern of the downstream gene product can then have concentration valleys where its inhibitor gene products have concentration peaks and concentration peaks where its activator gene products have concentration peaks (see section S5.4 of SI for further details). Similarly, a gene product that is positively downstream of gene products with different patterns would also develop a new non-trivial pattern in which concentrations peak would form wherever any of the upstream gene products have concentration peaks (Figure S4). The number of peaks could also be up to the sum of the number of peaks in the upstream gene products (even slightly more as explained in section S5.4 of SI). By hierarchically combining these positive and inhibitory interactions, complex patterns can be produced (Figure S4).

The ensemble of possible pattern transformations from H gene networks and spike initial conditions

For any non-trivial pattern transformation (as long as it is symmetric around the initial spike), there exists a H gene network capable of producing it from a spike initial pattern. To understand that let us first acknowledge that any resulting pattern can be seen as a sequence of concentration peaks of different heights and steepness at different positions. Now, let us consider the so-called diamond network. This simple gene network leads to a two-peak pattern around the initial spike (see **Figure 5**). By choosing the adequate parameters and f in this network, any combination of height, steepness and positions can be achieved in those peaks (see section S5.5 of SI). This means that by combining different diamond networks (i.e. by having several diamond networks upstream of the same gene product as explained in the previous section), it is possible to develop a pattern with any combination of peak height's, positions and steepnesses (that is any resulting pattern that is symmetric around the spike) (see section S5.6 in SI).

Pattern formation in H networks from homogeneous initial patterns

No non-trivial pattern transformations are possible in H gene networks from homogeneous initial patterns. In homogeneous initial patterns, the inhibition between gene products that are downstream from the signal would occur in the same way everywhere and, thus, it would not lead to non-trivial patterns (see section S5.7 of SI). The pattern transformations possible from the combined spike-homogeneous initial patterns are similar to those possible from the spike initial conditions (see section S5.1 of SI).

Emergent gene networks

L^+ gene networks

These are gene networks with one or several L^+ subnetworks downstream of a gene expressed in the initial pattern (and no other type of subnetwork). From a spike initial pattern, the signal, or signals, in the L^+ diffuse from the spike and activate their own production in the cells that receive it. As a result, the signal spreads further and ends up being produced by all cells (Figures S8-9). Due to the averaging effect of diffusion and the self-activating nature of L^+, all cells end up having the same concentration of the signal and, thus, the resulting pattern is homogeneous. The same occurs from homogeneous or spike-homogeneous combined initial patterns.

L^- gene networks

These networks only contain subnetworks with extracellular signals that inhibit their own production, directly or indirectly, in the cells that receive them (i.e., an L^- subnetwork). According to requirement I2, gene networks leading to non-trivial pattern transformations must include one or several self-activatory loops positively upstream of the genes expressed in the resulting pattern. In the case of L^- gene networks, these loops are intracellular (otherwise by our definition of gene network topologies, the network would be L^-L^+ not L^-). Let l^+_A denote a positive intracellular loop positively upstream of A.

Pattern transformations from homogeneous and spike initial patterns in L^- subnetworks

Let us first consider the case of an L^-gene network with a single L^- subnetwork and a single l^+_A that is positively upstream of L^-, but not downstream of it. From a homogeneous initial condition, and because requirement I1, the gene products in l^+_A will end up being expressed everywhere at the same level regardless of any noise present in the initial pattern since l^+_A is a positive

intracellular self-activatory loop. Hence, given that *A* is downstream of l^+_A, it will also be expressed everywhere at the same level, diffuse and inhibit itself by the same amount everywhere, i.e., no non-trivial pattern transformation (see Figures S8-9).

From a spike initial pattern there will be no non-trivial pattern transformation either. This is because there is no way to spread the expression of the genes in l^+ away from the spike since this would require an extracellular signal activating gene products in cells away from the spike but, by definition, the only existing extracellular signal inhibits itself. The same occurs if L^- is upstream of l^+_A but l^+_A is not upstream of L^- (Figures S8-9).

The only option left for non-trivial pattern transformations is that l^+_A is both upstream and downstream of *A*. This way, cells that extracellularly receive *A*, inhibit its production, while, intracellularly, *A* promotes its own production via l^+_A. We call these L^- gene networks, noise-amplifying gene networks (**Figure 4**), as in (Marcon *et al.*, 2016; Diego *et al.*, 2018).

It has been proven that noise-amplifying gene networks can transform homogeneous initial patterns into a specific type of non-trivial patterns (Marcon *et al.*, 2016; Diego *et al.*, 2018). Let us briefly summarize how these arise. Because of molecular noise, the homogeneous initial pattern consists of one or several gene products with random tiny concentration peaks and valleys over an otherwise homogeneous concentration level. Because of requirement I1 and I2, l^+_A is positively downstream of these gene products and, thus, acquires the same tiny concentration peaks and valleys l^+_A is a self-activatory loop and, thus, these peaks and valleys start to grow. *A* diffuses from each growing peak and, by the definition of L^-, it inhibits itself and in the cells receiving it (see Figure S6). Since A activates l^+_A, inhibiting *A* means inhibiting l^+_A too. As a result, the growing peaks of *A* and l^+_A inhibit the growth of other peaks of *A* and l^+_A nearby. Thus, there is a sort of lateral inhibition between peaks and the resulting pattern consist of concentration peaks (and valleys) that are further a part than in the initial pattern and are much taller (or deeper). The resulting patterns seem random but peaks are more likely to form where the initial pattern had higher tiny random peaks or where there were, by chance, fewer of them. In fact, for each distinct homogeneous initial pattern with random noise, a different resulting pattern can arise (see Figure S6). We call these resulting patterns, amplified noise patterns (as in Marcon 2016).

Combined spike-homogeneous initial patterns

As in the previous initial patterns, only the noise-amplifying gene networks can lead to non-trivial patterns transformations. Noise-amplifying gene networks are known to be able to lead to relatively complex and periodic non-trivial pattern transformations from spike-homogeneous initial conditions (Wang *et al.*, 2022). In this case, the concentration of *A* starts being higher in the spike than elsewhere. As *A* diffuses, it leads to the l^+_A being strongly inhibited in the cells around the spike and thus, to the formation of valleys of concentration for the gene products in l^+_A around it (see Figure S6B-C). Because l^+_A activates *A*, the cells in these valleys will produce less *A* and then, cells next to the valley will receive less *A*. As a consequence there will be less inhibition of l^+_A in the cells next to the valley and the concentration of both *A* and l^+_A will increase in those cells. This increase will, in turn, lead to the formation of a concentration valley further away and these valleys to further peaks and so on. This process continues until all the system is occupied by concentration peaks and valleys (of equal size and shape) (see Figure S6B-C). In 2D there are no peaks but concentric rings of high gene product concentration centered around the spike while in 3D there are concentric spherical shells. We refer to these patterns as 'frozen-wave' patterns. As in the case of H networks, these frozen wave patterns are symmetric around the initial spike but, in contrast to H networks, they extend over the whole system.

Changes in the parameters of noise-amplifying gene networks (e.g., in diffusivities), can lead to changes in the number, height or width of concentration peaks and valleys in the resulting pattern but, contrary to what happens for H gene networks, these peaks cannot change independently

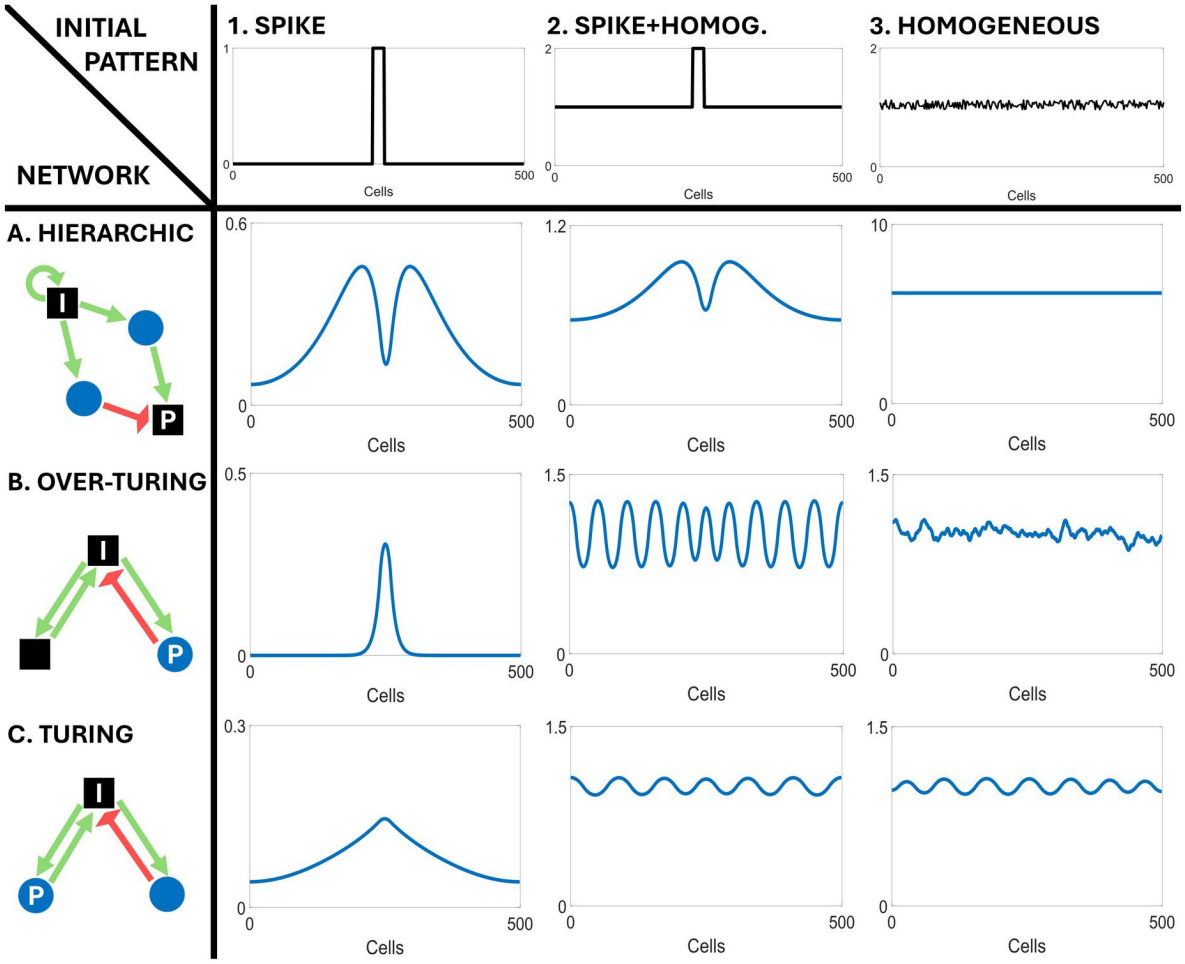


Figure 4

Gene networks capable of pattern formation and their resulting patterns.

The first column depicts simple examples of each type of gene network topology capable of non-trivial pattern transformation. The upper row shows the three initial patterns. Intermediate panels show each type of possible resulting pattern arising from each combination of initial pattern and gene network topology. Note that pattern transformations in **(B1), (C1)** and **(A3)** are trivial. Network colors and shapes as in Figure 2.*P*stands for the gene product plotted as resulting pattern while*I* stands for the gene product in the initial pattern. Simulations were run using a Forward-Euler algorithm on the Maini-Miura model (see S8 in SI for parameter values).

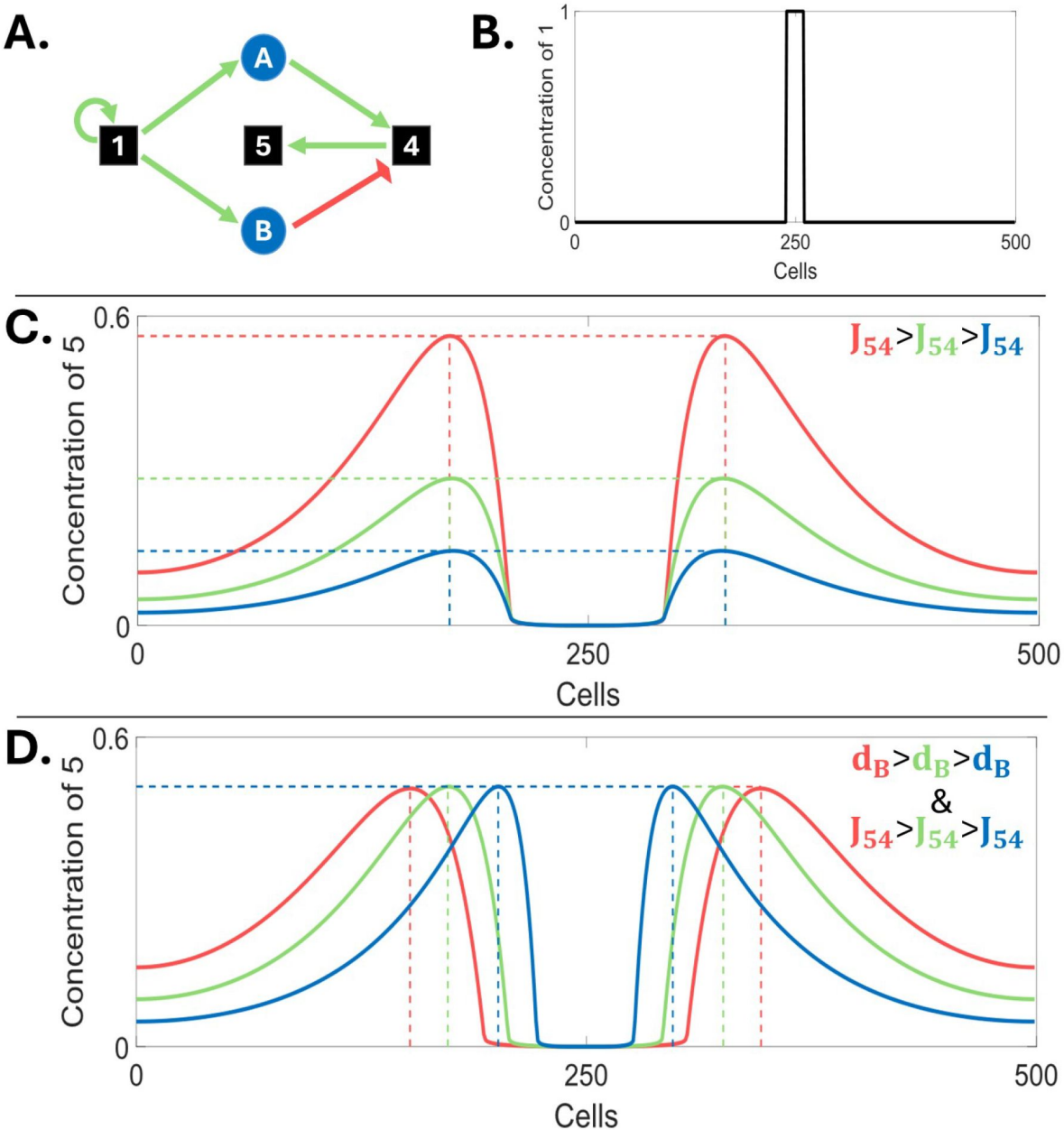


Figure 5

Variational properties of the diamond H network.

(A) Diamond H network (see S5.5 in SI). Network colors and shapes as in **Figure 2**. **(B)** Initial pattern in 1. **(C-D)** The resulting patterns consist in two symmetric peaks around the initial spike. The height **(C)** and position **(D)** of such peaks can be independently modified by tuning the model parameters. Simulations as in **Figure 4** (see S8 in SI for parameter values).

from each other (see Figure S7). This non-independence is easy to understand from the fact that noise-amplifying gene networks can produce many different concentration peaks from a small number of interactions in the gene network and thus, no specific part of the network is responsible for any specific concentration peak (as it happens for H gene networks). This implies that the ensemble of pattern transformations attainable by these networks are less diverse than the pattern transformations attainable by H gene networks (i.e., the former can only produce patterns with equally sized and shaped peaks, while the latter can produce any pattern that is symmetric around the spike).

Gene networks composed of several L^- subnetworks lead to the same patterns than L^- subnetworks and have very similar properties (see section S6.1 of SI).

Composite gene networks

Subnetworks can be combined in parallel, in series, or both. In parallel means that different subnetworks are upstream of the same set of gene products. Since in this case the gene networks do not regulate each other, it is easy to see that the resulting patterns in the most downstream genes can have concentration peaks and valleys wherever their upstream gene products have them, just as described for H^0 gene networks above (see Figure S4).

When combined in series, patterned gene products from one subnetwork are upstream of the extracellular signals of other subnetworks. The gene networks composed of L^+ upstream of H subnetworks are equivalent to H subnetworks acting on a homogeneous initial pattern (since we have seen that L^+ lead to homogeneous patterns) and, thus, lead to no non-trivial pattern transformation. If H is upstream of L^+, the attainable pattern transformations are those of H, since L^+ will homogenize the concentration of its constituent gene products (see section S5.4 of SI for details). More interesting pattern transformations can occur by combining noise-amplifying and H subnetworks acting on combined spike-homogeneous initial patterns in series. Simply, the frozen-wave pattern attainable by noise-amplifying gene network can be locally modified by the H subnetwork so that the peaks close to the initial spike can vary in size (symmetrically around the spike). This can happen both by the H being upstream of the noise-amplifying and the noise-amplifying being upstream of H (see Figure S10).

L^+L^- gene networks

If an L^+ subnetwork is upstream of an L^- subnetwork, and not downstream of it, L^+ is unaffected by L^- and consequently, its genes will be homogeneously expressed regardless of the initial pattern (see Figures S8-9). This reduces to what we have seen for L^- gene networks acting on homogeneous initial patterns (see Figures S8-9). If L^- is upstream of L^+, and not downstream of it, the genes in L^+cannot be expressed because the signal in L^-inhibits, by definition, other gene products in the loop (see Figures S8-9).

If L^+ and L^- are both upstream and downstream of each other, then we have the widely studied Turing mechanisms (Turing, 1952; Murray, 2002; Maini *et al*., 2006; Meinhardt, 2008). Turing mechanisms include an extracellular signal that promotes its own production (forming an L^+ subnetwork) and the production of another extracellular signal that inhibits the former (forming an L^- subnetwork). In Turing's seminal work, each subnetwork was in fact a single molecule (an extracelllarly diffusible one) but it has been widely reported that these mechanisms also apply to gene networks where extracellular signals regulate each other indirectly (though an intracellular part gene network (Salazar-Ciudad, *et al*. 2000; Satnoianu, *et al*., 2000; Maini *et al*., 2006; Meinhardt, 2008; Cotterell & Sharpe, 2010).

Turing gene networks can lead to non-trivial pattern transformation from homogeneous initial patterns (Turing, 1952) and from combined spike-homogeneous initial patterns (Tarumi & Mueller, 1989). The resulting patterns in all cases are very similar, as widely studied over the last 70 years (Turing, 1952; Maini *et al*., 2006; Meinhardt, 2008). These patterns consist of periodical, regularly spaced peaks and valleys of concentration that repeat themselves over space (**Figures 4** and S7). In 1D, these patterns are very similar to those possible by the noise-amplifying gene networks acting on spike-homogeneous initial patterns. In 2D and 3D, however, the resulting pattern consist of spots or stripes (Meinhardt, 1982). In contrast to H gene networks, the spatial information in the initial pattern is not used to construct the resulting pattern and hence, the same, or a very similar, resulting pattern emerges regardless of the initial pattern (Meinhardt, 1982).

As in the case of noise-amplifying gene networks, changes in the parameters of the gene network (e.g., in diffusivities), can change the number, height or width of concentration peaks and valleys in the resulting pattern and, contrary to H gene networks, no peaks or valleys can change their height or width independently from each other (Turing, 1952; Murray, 2002; Maini *et al*., 2006; Meinhardt, 2008). The reasons for this difference are the same we discussed for noise-amplifying gene networks (see Figure S7). This implies, again, that the ensemble of attainable pattern transformations by Turing gene networks is less diverse than the ensemble of attainable pattern transformations by H gene networks (Salazar-Ciudad, *et al*. 2001) (**Figure 6**).

Naturally, different Turing subnetworks can be combined into composite gene networks. If they are connected in parallel, there can be the same peak addition and subtraction we described for H networks (see Figure S4). By combining Turing subnetworks in series, the resulting pattern of one subnetwork can serve as the initial pattern of the other (Fujita & Kawaguchi, 2013; Moustakas-Verho *et al*., 2014). These combinations, however, do not lead to different kinds of patterns; the resulting patterns are still the periodic patterns that result from simple Turing networks (possibly combining several wavelengths as in Fujita & Kawaguchi, 2013 and Moustakas-Verho *et al*., 2014). Thus, combining different Turing gene networks does not lead to qualitatively different resulting patterns nor dynamics, as we show mathematically in section S6.2 of the SI.

Other combinations

In general, the Turing H combined gene networks have variational properties with features of both the Turing and H gene networks (Figure S10). Namely, peaks and valleys will form over the whole system (as in Turing gene networks) and the height of some of these peaks and valley can be varied independently by changes in the parameters of the H subnetwork. These combined properties have been discussed in detail before (Salazar-Ciudad et a., 2001; Miura, 2013; Green & Sharpe, 2015; Glim *et al*., 2021; Tzika *et al*., 2023).

From homogeneous initial patterns, combining Turing and noise-amplifying gene networks leads to resulting patterns in between those possible from Turing and noise-amplifying. Essentially periodic patterns with different amplifications of noise will occur (section S6.2 of SI).

Discussion

In this article we have shown that there are only three possible classes of subnetworks (H, L^+ and L^-) and that these can only be combined in three classes of gene networks (H, Turing and noise-amplifying) to lead to non-trivial pattern transformations. We have also show that gene networks in different classes lead to different types of pattern transformations and have different properties. In section S7 of SI we discuss how these results should hold even if cells have complex temporal internal dynamics (e.g., limit cycles).

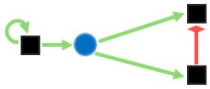
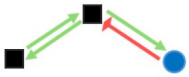
TYPE OF NETWORK	DO FINAL PATTERNS DEPEND ON INITIAL PATTERNS?	CAN PEAKS IN THE FINAL PATTERN BE CHANGED INDEPENDENTLY?	HOW HIGH IS THE DIVERSITY OF POSSIBLE FINAL PATTERNS?
Hierarchical networks 	**YES ✓** - Spike IP → Spiked pattern. - Sp.-Hom. IP → Spiked pattern. - Hom. IP → No pattern.	**YES ✓** Upstream gene products are unaltered by changes in downstream gene products.	**VERY HIGH ↑↑** A hierarchic network can be engineered to reproduce almost any sequence of peaks.
Over -Turing networks 	**YES ✓** - Spike IP → Trivial pattern. - Sp.-Hom. IP → Periodical pattern. - Hom. IP → Amplified noise.	**NO ✗** Changes in any gene product produce global changes in the final pattern.	**LOW ↓** In 1D, only noisy or periodical patterns. In higher dimensions, noisy or concentric rings.
Turing networks 	**NO ✗** - Spike IP → Trivial pattern. - Sp.-Hom. → Periodical pattern. -Hom. IP → Periodical pattern.	**NO ✗** Changes in any gene product produce global changes in the final pattern.	**HIGH ↑** In 1D, only periodical patterns. In higher dimensions, spots or stripes.

Figure 6

Some variational properties of the fundamental gene network topologies capable of pattern transformation.

Network colors and shapes as in **Figure 2**.

The fact that there are only three classes of gene networks leading to pattern transformation can be understood from simple qualitative geometric arguments. From a homogeneous initial pattern there is no way by which gene product concentrations could become different in one spatial part of the system and not on another unless these differences would be ultimately random (as in the noise-amplifying case) or regularly periodic over space (as in in the Turing case). From a spike initial pattern, gene product concentrations can become different in different parts of a pattern because, ultimately, these parts are at different distances from the spike and then exhibit different combinations of concentrations of the signals secreted from it. Based on these spatial differences specific parts of a H gene network can lead to different concentration peaks and valleys and their features (e.g., width, height, etc.).

The fact that there is a limited number of topological classes leading to pattern formation does not depend on the geometry of space (i.e., the distribution of cells in the embryo). The type of pattern transformations for each class does not depend on the geometry of space either, e.g., Turing networks lead to periodic patterns, H networks do not, etc. The geometry of space, however, can change specific aspects of these patterns (Diambra & Costa, 2006; Glimm *et al*., 2014; Castelino *et al*., 2020).

All the gene network topologies capable of pattern transformations that we describe have been individually reported before in one way or another (Turing, 1952; Salazar-Ciudad *et al*., 2001; Cotterell *et al*., 2015; Wang, 2022). What has not been shown before is that these are the only possible gene network topologies in pattern transformation, given the biologically reasonable restrictions on *f* that we describe. We think this is important both for theory and practice. For theory it is important because we provide a general description of the possible at the level of pattern transformations and underlying gene network topologies (as long as there is no cell movement). In practice this is also important because it provides a simple guideline for experimental developmental biologist trying to understand pattern transformations in specific organ over a set of developmental stages. Thus, if no cell movements occur during these stages, the underlying gene network should have a topology and variational properties among the ones described in this article. This narrows down significantly the range of possible underlying gene networks and patterning mechanisms to consider when trying to understand the development of a multicellular system. Similarly, this allows to better the study the evolution of gene networks leading to pattern transformations, since then the evolution of the development of any animal species can be understood as the tuning and replacing between these classes of gene networks along the sequence of development (Salazar-Ciudad *et al*., 2001).

Of the three classes of gene network topologies we identify, two have been widely studied before. Most of the research in Turing gene networks has been theoretical (Turing, 1952; Maini *et al*., 2006; Meinhardt, 2008) but there is also direct experimental research on the involvement of these gene networks in the development of many organs (e.g., Glover *et al*., 2017; 2023; Johnson *et al*., 2023; Tzika *et al*., 2023; Tseng *et al*., 2024, just to name some recent ones), although the underlying networks are usually more complex than the ones studied theoretically. Although hierarchical networks are usually not called this way, they constitute the bulk of the gene networks experimentally studied (Salazar-Ciudad *et al*., 2001) and they are, by far, the easier to understand. This, and the fact that many network seem hierarchical when only some of their interactions are known (Salazar-Ciudad, 2009), may have biased research to focus on them. Although noise-amplifying gene networks seem simpler and easier to understand than Turing networks, they have only been discovered very recently (Cotterell *et al*., 2015; Marcon *et al*., 2016; Wang *et al*., 2022). It is, thus, an open and suggestive question whether this class of network is widely used in pattern transformations in multicellular systems. In fact, it is perfectly possible that organs that are though to use Turing networks may actually be using noise-amplifying networks instead, since the latter produce resulting patterns that are very similar, specially when the organ is effectively 1D.

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Additional files

Supporting information

Additional information

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Editors

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Reviewer #1 (Public review):

Summary:

The authors tackle a long-standing question in developmental theory: given a gene-regulatory network that includes extracellular signalling, which topologies are even capable of transforming an initial spatial profile into a genuinely new pattern? Building on the classical reaction-diffusion framework in one dimension, but imposing biologically motivated constraints, they prove that every one-signal sub-network must be either Hierarchical (H), self-activating (L+), or self-inhibiting (L-). They further demonstrate that only three composite classes of full networks - pure H, a coupled L+ L- "Turing" pair, and an L- module fed by an intracellular positive loop ("noise-amplifying")-can create non-trivial spatial transformations. Analytical criteria and illustrative simulations are provided, together providing a closed taxonomy, which is supposed to be relevant for real systems.

Strengths:

(1) Useful classification framework. Reducing a vast number of possible gene circuits to three canonical pattern-forming motifs is a valuable organising insight for both theorists and experimentalists.

(2) Logical completeness. All required cases are addressed, and the proofs elevate previous computational observations to formal statements.

(3) Practical interpretability. Given a reaction network diagram, one can now decide (assuming the model applies to the real systems) whether spatial patterning is even possible, saving experimental effort on in-silico screens that could never succeed.

Weaknesses:

(1) The Results section is difficult to follow. Key logical steps and network configurations are described shortly in prose, which constantly require the reader to address either SI or other parts of the text (see numerous links on the requirements R1-R5 listed at the beginning of the paper) to gain minimal understanding. As a result, a scientifically literate but non-specialist reader may struggle to grasp the argument with a reasonable time invested.

(2) A central step in the model formulation is the linearisation of the reaction term around a homogeneous steady state; higher-order kinetics, including ubiquitous bimolecular sinks such as $A + B \rightarrow AB$, are simply collapsed into the Jacobian without any stated amplitude bound on the perturbations. Because the manuscript never analyses how far this assumption can be relaxed, the robustness of the three-class taxonomy under realistic nonlinear reactions or large spike amplitudes remains uncertain.

(3) All modelling is confined to one spatial dimension, and the very definition of a "non-trivial" transformation is framed in terms of peak positions along a line, which clearly must be reformulated for higher dimensions. It's well-known that diffusions in 1, 2, and 3 dimensions are also dramatically different, so the relevance of the three-class taxonomy to real multicellular tissues remains unclear, or at least should be explained in more detail.

Discussion:

As stated above, there are several uncertainties about the relevance of the presented framework for real systems. However, if the results hold, researchers could look at a gene-network diagram and quickly judge whether it can make spatial patterns and, if so, which of the three known mechanisms it will use. That shortcut would save experimental and computational time. In the case that the results don't hold for the real systems, the authors' proof tools at least give theorists a solid base they can extend to more complex cases.

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Reviewer #2 (Public review):

Summary:

This study explores how gene regulatory networks that include intra- and extracellular signaling can give rise to spatial patterns of gene expression in cells. The authors investigate this question in a simplified theoretical framework, where all cells are assumed to respond identically to signals, and spatial details such as cell boundaries and extensions are abstracted away. Within this setting, they identify three distinct signaling topologies, referred to as L and H types, and combine them into three minimal subnetworks capable of generating patterns. The study analyzes possible combinations of these topologies and examines how each subnetwork behaves under three different initial conditions. Combining the analyses with mathematical proofs and heuristic arguments, the authors define necessary conditions under which such networks can produce non-trivial spatial patterns.

Strengths:

The authors break down larger gene regulatory networks into smaller subnetworks, which allows for a more tractable analysis of pattern formation. These minimal subnetworks are examined under different initial conditions, providing a range of examples for how patterns can emerge in simplified settings. The study also proposes necessary conditions for pattern formation, which may be useful for identifying relevant network structures. In addition, the manuscript offers heuristic explanations for the emergence of patterns in each subnetwork, which help to interpret the simulation results and analytical criteria.

Weaknesses:

(1) We have serious concerns regarding the validity of the simulation results presented in the manuscript. Rather than simulating the full nonlinear system described by Equation (1), the authors base their results on a truncated expansion (Equation S.8.2) that captures only the time evolution of small deviations around a spatially homogeneous steady state. However, it remains unclear how this reduced system is derived from the full equations - specifically, which terms are retained or neglected and why - and how the expansion of the nonlinear function can be steady-state independent, as claimed. Additionally, in simulations involving the spike plus homogeneous initial condition, it is not evident - or, where equations are provided, it is not correct - that the assumed global homogeneous background actually corresponds to a steady state of the full dynamics. We elaborate on these concerns in the following:

It is assumed that the homogeneous steady states are given by g_i=0 and g_i=c_i, where 1/c_i = \mu_i or \hat{\mu}_i, independently of the specific network structure. However, the basis for this assumption is unclear, especially since some of the functions do not satisfy this condition - for example, f5 as defined below Eq. S8.10.5. Moreover, if g_i=c_i does not correspond to a true steady state, then the time evolution of deviations from this state is not correctly described by Eq. S8.2, as the zeroth-order terms do not vanish in that case.

Additionally, the equations used contain only linear terms and a cubic degradation term for each species g_i, while neglecting all quadratic terms and cubic terms involving cross-species interactions (i≠j). An explanation for this selective truncation is not provided, and without knowledge of the full equation (f), it is impossible to assess whether this expansion is mathematically justified. If, as suggested in the Supplementary Information, the linear and cubic terms are derived from f, then at the very least, the Jacobian matrix should depend on the background steady-state concentration. However, the equations for the small deviation around a steady state (including the Jacobian matrix) used in the simulations appear to be independent of the particular steady state concentration.

This is why we believe that the differences observed between the spike-only initial condition and the spike superimposed on a homogeneous background are not due to the initial conditions themselves, but rather result from a modified reaction scheme introduced through a questionable cutoff.

"In simulations with spike initial patterns, the reference value g≡0 represents an actual concentration of 0 and therefore, we must add to (S8.2) a Heaviside function Φ acting of f (i.e., Φ(f(g))=f(g) if f(g)>0 , Φ(f(g))=0 if f(g){less than or equal to}0) to prevent the existence of negative concentrations for any gene product (i.e., g_i<0 for some i)." (SI chapter S8).

This cutoff alters the dynamics (no inhibition) and introduces a different reaction scheme between the two simulations. The need for this correction may itself reflect either a problem in the original equations (which should fulfill the necessary conditions and prevent negative concentrations (R4 in main text)) or the inappropriateness of using an expanded approximation which assumes independence on the steady state concentration. It is already questionable if the linearized equations with a cubic degradation term are valid for the spike initial conditions (with different background concentration values), as the amplitude of this perturbation seems rather large.

Lastly, we note that under the current simulation scheme, it is not possible to meaningfully assess criteria RH2a and RH2b, as they rely on nonlinear interactions that are absent from the implemented dynamics.

(2) Most of the proofs presented in the Supplementary Information rely on linearized versions of the governing equations, and it remains unclear how these results extend to the fully nonlinear system. We are concerned that the generality of the conclusions drawn from the linear analysis may be overstated in the main text. For example, in Section S3, the authors introduce the concept of dynamic equivalence of transitive chains (Proposition S3.1) and intracellular transitive M-branching (Proposition S3.2), which pertains to the system's steady-state behavior. However, the proof is based solely on the linearized equations, without additional justification for why the result should hold in the presence of nonlinearities. Moreover, the linearized system is used to analyze the response to a "spike initial pattern of arbitrary height C" (SI Chapter S5.1), yet it is not clear how conclusions derived from the linear regime can be valid for large perturbations, where nonlinear effects are expected to play a significant role. We encourage the authors to clarify the assumptions under which the linearized analysis remains valid and to discuss the potential limitations of applying these results to the nonlinear regime.

(3) Several statements in the main text are presented without accompanying proof or sufficient explanation, which makes it difficult to assess their validity. In some cases, the lack of justification raises serious doubts about whether the claims are generally true. Examples are:

"For the purpose of clarity we will explain our results as if these cells have a simple arrangement in space (e.g., a 1D line or a 2D square lattice) but, as we will discuss, our results shall apply with the same logic to any distribution of cells in space." (Main text l.145-l.148).

"For any non-trivial pattern transformation (as long as it is symmetric around the initial spike), there exists an H gene network capable of producing it from a spike initial pattern." (Main text l.366f).

"In 2D there are no peaks but concentric rings of high gene product concentration centered around the spike, while in 3D there are concentric spherical shells." (Main text l. 447ff).

(4) The study identifies one-signal networks and examines how combinations of these structures can give rise to minimal pattern-forming subnetworks. However, the analysis of

the combinations of these minimal pattern-forming subnetworks remains relatively brief, and the manuscript does not explore how the results might change if the subnetworks were combined in upstream and downstream configurations. In our view, it is not evident that all possible gene regulatory networks can be fully characterized by these categories, nor that the resulting patterns can be reliably predicted. Rather, the approach appears more suited to identifying which known subnetworks are present within a larger network, without necessarily capturing the full dynamics of more complex configurations.

(5) The definition of non-trivial pattern formation is provided only in the Supplementary Information, despite its central importance for interpreting the main results. It would significantly improve clarity if this definition were included and explained in the main text. Additionally, it remains unclear how the definition is consistently applied across the different initial conditions. In particular, the authors should clarify how slope-based measures are determined for both the random noise and sharp peak/step function initial states. Furthermore, the authors do not specify how the sign function is evaluated at zero. If the standard mathematical definition sgn(0)=0 is used, then even a simple widening of a peak could fulfill the criterion for non-trivial pattern transformation.

(6) The manuscript lacks a clear and detailed explanation of the underlying model and its assumptions. In particular, it is not well-defined what constitutes a "cell" in the context of the model, nor is it justified why spatial features of cells - such as their size or boundaries - can be neglected. Furthermore, the concept of the extracellular space in the one-dimensional model remains ambiguous, making it unclear which gene products are assumed to diffuse.

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Reviewer #3 (Public review):

Pattern formation is responsible for generating the spatial organization of cells, tissues, and organs during embryogenesis. It operates within a multifactorial system including initial conditions, gene regulatory networks, extracellular signals, mechanical forces, stochastic noise, and environmental inputs. Finally, it ensures the functional anatomy of an organism.

This study focuses on the one central aspect in pattern formation: how spatial heterogeneity arises from an initial condition and evolves into a more complex or distinct spatial pattern (non-trivial pattern formation, as they termed). The authors made efforts to explore and characterize all possible ways to achieve the pattern formation. They do this by discussing how extracellular signals spread, how individual cells respond to those signals, and how those responses, in turn, modulate signal propagation.

Finally, their comprehensive analysis summarizes that there are three classes of interactions between extracellular signals and intracellular responses, corresponding to previously known mechanisms that can generate spatial patterns: difference in morphogen concentrations in space, noise-amplification, and Turing pattern.

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Author response:

We thank the reviewers for their thorough evaluation and constructive feedback on our manuscript.

We think that their valuable suggestions will strengthen the manuscript and help us clarify several important points.

All reviewers acknowledged the importance of our theoretical results and network classification in making pattern formation analysis a more tractable problem. At the same time, they have also raised a number of important concerns that we shall carefully consider.

A. A major clarification that the reviewers found important concerns the definition of non-trivial pattern transformations and its generalization to higher dimensions. In this regard, the reviewers' comments are:

> ***Reviewer #1:***
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> *(on non-trivial pattern transformations):*
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> *(3) All modelling is confined to one spatial dimension, and the very definition of a "non-trivial" transformation is framed in terms of peak positions along a line, which clearly must be reformulated for higher dimensions. It's well-known that diffusions in 1, 2, and 3 dimensions are also dramatically different, so the relevance of the three-class taxonomy to real multicellular tissues remains unclear, or at least should be explained in more detail. Reviewer #2 (on non-trivial pattern transformations):*
>
> *(5) The definition of non-trivial pattern formation is provided only in the Supplementary Information, despite its central importance for interpreting the main results. It would significantly improve clarity if this definition were included and explained in the main text. Additionally, it remains unclear how the definition is consistently applied across the different initial conditions. In particular, the authors should clarify how slope-based measures are determined for both the random noise and sharp peak/step function initial states. Furthermore, the authors do not specify how the sign function is evaluated at zero. If the standard mathematical definition sgn(0)=0 is used, then even a simple widening of a peak could fulfill the criterion for nontrivial pattern transformation.*

We agree with Reviewer #2 that including a more detailed definition of non-trivial pattern transformation in the main text would enhance the clarity of the paper. The one-dimensional (1D) definition currently provided in the Supplementary Information was chosen because all computations presented therein involve exclusively one-dimensional patterns. However, we acknowledge that this definition, as it was, did not have a totally unambiguous generalization to higher dimensions. Therefore, in a revised version of the manuscript, we will incorporate an expanded definition applicable to higher-dimensional cases.

This general definition of a non-trivial pattern transformation should make no reference to the sign of spatial derivatives of either the initial or resulting patterns. Specifically, a pattern transformation is considered non-trivial if it satisfies the following criteria:

- It is heterogeneous: The resulting pattern is heterogeneous in space.

- It is rearranging: The arrangement of critical points (i.e. peaks, valleys and saddle points in a gene product concentration) along the domain in the resulting pattern of a gene product is different to the arrangement of critical points in its initial pattern. This includes the emergence of new critical points, the disappearance of existing ones, or the spatial displacement of critical points from one location to another.

- It is non-replicating: The spatial arrangement of critical points in the pattern of one gene product must differ from that of any other upstream gene product.

Nonetheless, our two initial patterns are spatially discontinuous functions: in homogeneous initial patterns, the white noise is discontinuous by definition; and for the spike and spike+homogeneous initial patterns, we use sharp spikes defined by the rectangular function,

which is discontinuous at the spike boundaries. Therefore, the aforementioned definition should be supplemented with the following two *ad hoc* assumptions:

- Homogeneous initial patterns do not comprise any critical point. White noise in this type of initial patterns represents small thermodynamic fluctuations around the steady state and, for the purpose of pattern transformation, this is equivalent to a constant concentration along the domain.

- Spike and spike+homogeneous initial patterns each contain a single critical point located at the center of the spike. The sharp spikes, modeled using the rectangular function, serve as a theoretical idealization to facilitate mathematical analysis. Once diffusion begins to act, these sharp boundaries are smoothed into differentiable gradients, maintaining a unique critical point at the center of the initial spike, which is the most relevant information for pattern transformation.

Finally, it is worth recalling that our gene network classification is fundamentally based on an analysis of the dispersion relation associated with the gene network, and the construction of this dispersion relation is independent of the spatial dimensionality of the domain (i.e. it does not require assuming any specific number of dimensions). The fact that the description of this dispersion relation was in the SI may have been non-ideal for the understandability of the article and will, consequently, be moved to the main text in an upcoming version of the article. Thus, the gene networks that can lead to pattern transformation are the same in 1D, 2D or 3D. As for the resulting patterns, the broad description we provide also applies to any number of dimensions; these would be periodic, non periodic as in the amplified noise patterns or non periodic as in the hierarchic networks. For the latter notice that, except for boundary effects that we later discuss, the spike initial condition is radially symmetric and thus, the patterns resulting from it will also be radially symmetric. We will make this point more explicit in a revised version of the article, especially since, as suggested, this important portion of the Supplementary Information will be incorporated into the main text.

Reviewer 2 suggests that with our definition of non-trivial pattern transformation, the simple widening of a concentration peak would constitute a non-trivial pattern transformation. This is not the case, as already shown in the figures as a example, since in a widening there is no change in the position of the critical point. A different situation applies if a wide and completely flat concentration peak (i.e. a plateau) forms. As we will explain in the coming version this is not possible because of requirement R5.

We think that this clarification of the definition of non-trivial pattern transformation will also help clarify the next point (B below) since it would make it clearer that this article does not intend to explain which specific resulting pattern would arise from any given gene network.

B. The main concern among these relates to the validity of our linearization of the model equations and the extension of the results obtained for the linear system to the fully nonlinear system. In this regard, the reviewers' comments are:

> ***Reviewer #1:***
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> *(on linearization):*
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> *(2) A central step in the model formulation is the linearisation of the reaction term around a homogeneous steady state; higher-order kinetics, including ubiquitous bimolecular sinks such as A + B → AB, are simply collapsed into the Jacobian without any stated amplitude bound on the perturbations. Because the manuscript never analyses how far this assumption can be relaxed, the robustness of the three-class taxonomy under realistic nonlinear reactions or large spike amplitudes remains uncertain.*

> ***Reviewer #2:***
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> *(on linearization):*
>
> *(2) Most of the proofs presented in the Supplementary Information rely on linearized versions of the governing equations, and it remains unclear how these results extend to the fully nonlinear system. We are concerned that the generality of the conclusions drawn from the linear analysis may be overstated in the main text. For example, in Section S3, the authors introduce the concept of dynamic equivalence of transitive chains (Proposition S3.1) and intracellular transitive M-branching (Proposition S3.2), which pertains to the system's steady-state behavior. However, the proof is based solely on the linearized equations, without additional justification for why the result should hold in the presence of nonlinearities. Moreover, the linearized system is used to analyze the response to a "spike initial pattern of arbitrary height C" (SI Chapter S5.1), yet it is not clear how conclusions derived from the linear regime can be valid for large perturbations, where nonlinear effects are expected to play a significant role. We encourage the authors to clarify the assumptions under which the linearized analysis remains valid and to discuss the potential limitations of applying these results to the nonlinear regime.*

In this article, we address two main questions: first, which gene network topologies can give rise to non-trivial pattern transformations; and second, which broad types of resulting patterns can these gene network topologies give rise to resulting pattern. Thus, we are not intending to explain which exact resulting patterns would arise from any given gene network (i.e. a gene network topology with specific functions and interaction strengths or weights), a question for which non-linearities do indeed matter.

For most known gene regulatory networks, available empirical information is typically limited to the nature of gene product regulations -indicating whether they act as activators or inhibitors- while details about the specific functional form of these regulations are rare. For instance, given two gene products, *i* and *j*, the network may indicate that *i* acts as an activator of *j*, implying that the concentration of *j* increases with that of *i*. However, this increase could follow a variety of functional forms: it may be quadratic (e.g., $\partial_t g_j = C g_i^2$), cubic (e.g., $\partial_t g_j = C g_i^3$), or any other function $f_j(g_i)$. As we explain in the description of our model, we restrict our study to functions with a monotonicity constraint: higher concentrations of *i* lead to increased production of *j* (i.e., $\partial_{g_i} f_j(g_i) > 0$). In other words, a given gene interaction is always inhibitory or activatory, it does not change of sign. This monotonicity constraint corresponds to requirement (R5) in our main text. This requirement it is based on the biologically plausible idea that the complexity of gene regulation in development stems more from the topology of gene networks than from the complexity of the regulation by which a gene product may regulate another (i.e. we use simple monotonic functions).

Question 1: A critical part to understand question 1 is in the dispersion relation that was explained in SI. From the reviewers' comments it is clear that having this crucial part in the main text of an upcoming version of the article would improve understandability, specially for question 1.

In brief, any pattern transformation requires the initial pattern to change. The trigger of such change is a change in the concentration of some gene product, either conceptualized as a noise fluctuation (in the homogeneous initial pattern) or a regulated change in a specific point (in the spike initial pattern). Mathematically, both can be conceptualized as

perturbations and, for pattern transformation to be possible, such perturbation should grow so that the initial pattern becomes unstable and can change to another resulting pattern.

If the perturbation is small, one can use the standard linear perturbation analysis in S6.2 of our Supplementary Information. In other words, the linear analysis is enough to ascertain if a small perturbation would grow or not. A gene network in which this will not happen would be unable to lead to pattern transformation, whichever the nonlinear part of $\boldsymbol{f}(\boldsymbol{g})$. In that sense, the linear approximation provides a necessary condition that any gene network needs to fulfill to lead to pattern transformation.

However, the linear analysis would not ascertain whether a specific gene network will actually lead to pattern transformation (i.e., the condition is not sufficient). This, as well as the shape of the specific resulting pattern, may actually depend on the non-linear parts too. As we discuss, based on the dispersion relation, and other complementing arguments along the article, we can also get some insights on the possible patterns from the linear approximation alone (question 2). This arguments hold thanks to the imposition of requirements (R1-R5) on function $\boldsymbol{f}(\boldsymbol{g})$, which prevent strange behaviors stemming from the nonlinear part of the equation.

The amplitude bound of perturbations mentioned by Reviewer #1 is addressed by requirements (R2) and (R4). Although the solution to the linear system predicts unbounded growth of unstable eigenmodes, the assume functions $\boldsymbol{f}(\boldsymbol{g})$ on which the nonlinear terms eventually halt this growth, thereby ensuring the boundedness of solutions as imposed by (R4). This assumption on the nonlinear part is literally requirement R2 on $\boldsymbol{f}(\boldsymbol{g})$ in the main text.

The transitive chains and branchings in section S3 of the Supplementary Information mentioned by the Reviewer #2 are topological properties of gene networks and therefore they influence only the linear part of the reaction-diffusion equations. This is why the proofs in that section are based on the linearized equations. We agree that clarifying this point in the text, as suggested by the reviewer, would improve the reader's understanding of the section.

Regarding Reviewer #2's concerns about large perturbations, we acknowledge that the phrasing using "arbitrary height" may be confusing. For the homogeneous initial conditions these perturbations are assumed to be small because they are actually molecular noise (otherwise the initial condition could not be considered homogenous in the classical sense of developmental biology models). In the spike initial conditions in hierarchic networks the perturbation is not necessarily small. For the analysis provided in the SI we indeed assume that the perturbations are small enough for the linear approximation to be possible. Notice, however, that since these networks require an intracellular self-activating loop upstream of the first extracellular signal, the effective perturbation would rapidly grow to a value determined by such loop.

In general the height of the initial spike does not affect the fact that hierarchic networks can lead to non-trivial pattern transformation. By definition these networks require the secretion of an extracellular signal from the cells in the spike (otherwise no change in gene product concentrations can occur over space). By definition this signal is not produced by any other cells and, thus, its concentration is governed by diffusion from the spike and its production in the cells in the spike. Thus, whichever the initial height of the spike and whichever the non-linearities in $\boldsymbol{f}(\boldsymbol{g})$, the signal's concentration would decrease with the distance from the spike. As explained in the main text, this would lead to non-trivial pattern transformations if other general conditions are met. In general, the height of the initial perturbation can affect which specific pattern transformation would arise from a specific gene network but not which gene network topologies can lead to pattern transformation. This will be more clearly stated in an upcoming version of the article. C. In the following, we respond to the remaining concerns raised by the reviewers:

> ***Reviewer #1:***
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> *(1) The Results section is difficult to follow. Key logical steps and network configurations are described shortly in prose, which constantly require the reader to address either SI or other parts of the text (see numerous links on the requirements R1-R5 listed at the beginning of the paper) to gain minimal understanding. As a result, a scientifically literate but non-specialist reader may struggle to grasp the argument with a reasonable time invested.*

We acknowledge that the current version of the main text may not be as clear as we intended. Initially, we believed that placing the more technical mathematical passages in the Supplementary Information would make the main text more accessible to readers. However, we agree with the reviewer that including some of these computations in the main text could improve clarity. We also believe that adding a summary table outlining all the model's requirements would further contribute to that goal.

> ***Reviewer #2:***
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> *(1) We have serious concerns regarding the validity of the simulation results presented in the manuscript. Rather than simulating the full nonlinear system described by Equation (1), the authors base their results on a truncated expansion (Equation S.8.2) that captures only the time evolution of small deviations around a spatially homogeneous steady state. However, it remains unclear how this reduced system is derived from the full equations specifically, which terms are retained or neglected and why- and how the expansion of the nonlinear function can be steady-state independent, as claimed. Additionally, in simulations involving the spike plus homogeneous initial condition, it is not evident -or, where equations are provided, it is not correct- that the assumed global homogeneous background actually corresponds to a steady state of the full dynamics. We elaborate on these concerns in the following:*

We believe there has been a misunderstanding regarding the presentation of the model equations (S8.2) used throughout our simulations. Accordingly, we agree that this relevant section of the Supplementary Information should be rewritten in a revised version of the manuscript to clarify this issue. Below, we address all the concerns raised by the reviewer.

Equation (S8.2) represents the full nonlinear system described in Equation (1). While we recognize that the model may oversimplify real biological processes, its purpose is to illustrate our general statements about pattern formation rather than to capture any specific or detailed mechanism. In this context, model (S8.2) offers three key advantages for our goals: it allows rapid manipulation of gene network topology simply by modifying the matrix J, making it ideal for illustrating pattern formation across different network classes; it accommodates gene networks of arbitrary size -unlike other models, such as the classical Gierer-Meinhardt model, which are limited to two-element Turing or noise-amplifying networks-; and, due to the simplicity of its nonlinear terms, this model involves relatively few free parameters, facilitating the fine-tuning needed to identify parameter regions where non-trivial pattern transformations occur.

Indeed, we find that the ability of model (S8.2) to illustrate our results despite having such simple nonlinear terms -bearing in mind that at least some nonlinearity is always necessary for selforganization- strongly supports the claim that the capacity of a gene network to produce pattern transformations is fully determined by the linear part of Equation (1). In this sense, nonlinear terms primarily influence the precise parameter values at which these transformations occur and contribute to shaping specific features of the resulting patterns.

Model (S8.2) has been successfully employed in pattern formation studies elsewhere in the literature; accordingly, we provide relevant bibliographic references to support its widespread use.

We believe the misunderstanding arises from our explanation of the biological interpretation of the model. As noted in the accompanying bibliography, the model is based on a general reactiondiffusion mechanism assuming the existence of a steady state. However, this conceptual reactiondiffusion framework is not the same as our Equation (1); rather, it was introduced by the original proponents of the model in the seminal paper cited in our text. In this context, Equation (S8.2) describes small concentration perturbations around that steady state, where the variables represent deviations in concentration relative to the general steady state.

The aforementioned general steady state corresponds to the trivial equilibrium point $g \equiv 0$ in equations (S8.2). Consequently, all our simulations based on model (S8.2) start from this steady state, to which we add white noise to generate homogeneous initial patterns or a sharp spike for the two types of spike initial patterns.

It is also worth noting that Equations (S8.2) represent a non-dimensional model.

> *It is assumed that the homogeneous steady states are given by g_i=0 and g_i=c_i, where 1/c_i = \mu_i or \hat{\mu}_i, independently of the specific network structure. However, the basis for this assumption is unclear, especially since some of the functions do not satisfy this condition -for example, f5 as defined below Eq. S8.10.5. Moreover, if g_i=c_i does not correspond to a true steady state, then the time evolution of deviations from this state is not correctly described by Eq. S8.2, as the zeroth-order terms do not vanish in that case.*

From the explanations above, it is important to distinguish two scales in the process: the scale of small perturbations, where equations (S8.2) apply; and the global scale, where the conceptual general reaction-diffusion system operates. Since the specific form of this general system does not affect equations (S8.2), we assume that it follows any of the models cited in the text, which yield a non-zero steady state at μ_i^{-1}.

In this sense, Equation (S8.2) represent a small concentration deviation of such global system and $g(t, x)$ is a relative concentration where $g \equiv 0$ represents the steady-state at μ_i^{-1}, $g>0$ are concentrations above μ_i^{-1}, and $g<0$ are concentrations below μ_i^{-1}.

As previously mentioned, simulations are performed using Equations (S8.2) on the basis of the equilibrium point $g \equiv 0$. The result of these simulations is then superimposed on the non-zero steady state μ_i^{-1} and presented in the figures along the article.

Using the full model instead of the simplified Equations (S8.2) may result in slightly different resulting patterns, but it does not affect the gene network's ability to produce pattern transformations, nor does it alter the main structural properties of the patterns—for example, the periodic nature of patterns generated by Turing networks.

> *Additionally, the equations used contain only linear terms and a cubic degradation term for each species g_i, while neglecting all quadratic terms and cubic terms involving cross-species interactions (i≠j). An explanation for this selective truncation is not provided, and*

> *without knowledge of the full equation (f), it is impossible to assess whether this expansion is mathematically justified. If, as suggested in the Supplementary Information, the linear and cubic terms are derived from f, then at the very least, the Jacobian matrix should depend on the background steady-state concentration. However, the equations for the small deviation around a steady state (including the Jacobian matrix) used in the simulations appear to be independent of the particular steady state concentration.*

The Jacobian of Equation (S8.2) is independent of g because g represents a small perturbation around a steady state of a general reaction-diffusion system. Consequently, the matrix J corresponds to the Jacobian of the general system evaluated at that steady state. Evaluating the Jacobian of equations (S8.2) at the equilibrium point $g \equiv 0$ -which represents the general steady state- recovers the matrix J.

> *This is why we believe that the differences observed between the spike-only initial condition and the spike superimposed on a homogeneous background are not due to the initial conditions themselves, but rather result from a modified reaction scheme introduced through a questionable cutoff.*
>
> *"In simulations with spike initial patterns, the reference value g≡0 represents an actual concentration of 0 and therefore, we must add to (S8.2) a Heaviside function Φ acting of f (i.e., Φ(f(g))=f(g) if f(g)>0 , Φ(f(g))=0 if f(g){less than or equal to}0) to prevent the existence of negative concentrations for any gene product (i.e., g_i<0 for some i)." (SI chapter S8).*
>
> *This cutoff alters the dynamics (no inhibition) and introduces a different reaction scheme between the two simulations. The need for this correction may itself reflect either a problem in the original equations (which should fulfill the necessary conditions and prevent negative concentrations (R4 in main text)) or the inappropriateness of using an expanded approximation which assumes independence on the steady state concentration. It is already questionable if the linearized equations with a cubic degradation term are valid for the spike initial conditions (with different background concentration values), as the amplitude of this perturbation seems rather large.*

For homogeneous and spike+homogeneous initial conditions, we interpret equations (S8.2) as small perturbations around a non-zero steady state of a general reaction-diffusion system. For spike-only initial conditions, that steady state is zero. As we mention before, $g \equiv 0$ will then represent such steady-state of zero concentration, $g>0$ are positive concentrations of the general system, and $g<0$ would represent unfeasible negative concentrations of the general system. Therefore, the use of a cutoff function to handle such initial conditions is justified. Moreover, this cutoff function is the same as the one employed in the reference general system cited in our paper.

We acknowledge that the cutoff influences the simulations and accounts for the differences observed between spike and spike+homogeneous initial conditions. However, this distinction reflects what occurs in real biological systems, which is precisely why we differentiate these two types of initial states. For instance, the emergence of a periodic pattern in a noise-amplifying network depends critically on the formation of regions with concentrations below the steady state near the initial spike. Such regions can form in spike-plus-homogeneous initial patterns but not in spike-only initial patterns, where concentrations below the steady state would correspond to biologically unfeasible negative values.

> *Lastly, we note that under the current simulation scheme, it is not possible to meaningfully assess criteria RH2a and RH2b, as they rely on nonlinear interactions that are absent from the implemented dynamics.*

It is explicitly stated in the relevant subsections of Section S7 in the Supplementary Information that, for the simulations involving RH2a and RH2b, the function $f(g)$ in equation (S8.2) is modified by adding an ad hoc quadratic term to enable the assessment of these criteria.

> *(3) Several statements in the main text are presented without accompanying proof or sufficient explanation, which makes it difficult to assess their validity. In some cases, the lack of justification raises serious doubts about whether the claims are generally true. Examples are:*
>
> *"For the purpose of clarity we will explain our results as if these cells have a simple arrangement in space (e.g., a 1D line or a 2D square lattice) but, as we will discuss, our results shall apply with the same logic to any distribution of cells in space." (Main text l.145-l.148).*

We believe that the confusion in this statement arises from the ambiguous use of the phrase “our results”. We will revise the text to provide a more precise description. Specifically, by “our results,” we refer to the conclusion that it is possible to determine whether a gene network leads to nontrivial pattern transformations based solely on its topology. This conclusion is independent of the dimensionality of space, as none of our arguments rely on assumptions specific to spatial dimensions. While one-dimensional examples are used for clarity and illustration, the underlying reasoning applies generally. In an improved version of the article, we will clarify this point explicitly and move relevant arguments from the Supplementary Information into the main text.

Critically, our classification of gene networks is ultimately based on an argument concerning the dispersion relation associated with the network, and the construction of this dispersion relation is independent of the spatial dimensionality of the domain. In this sense, the networks identified in the text as capable of producing pattern transformations will be able to generate non-trivial pattern transformations in any spatial domain and in any number of dimensions. While the specific parameter values that permit such transformations may vary depending on the geometry and dimensionality of the domain, the existence of at least one such parameter set remains unaffected.

The geometry of the domain can influence the specific form of the resulting patterns, but it does not alter the broader class of patterns (e.g., periodic patterns, peaks emerging around a spike, etc.) that a given gene network topology can produce. One such geometric influence, commonly observed in simulations, involves boundary effects. For example, structures such as peaks or rings forming near the boundaries may appear higher, broader, or spatially shifted compared to those arising in the central regions of the domain. However, we think a pattern consisting of a periodic train of peaks where only those near the boundary are slightly different can still be classified as a periodic pattern.

> *"For any non-trivial pattern transformation (as long as it is symmetric around the initial spike), there exists an H gene network capable of producing it from a spike initial pattern." (Main text l.366f).*

A justification for this statement is provided shortly after the claim, although we acknowledge that the current explanation is somewhat cumbersome and would benefit from a clearer presentation in a revised version of the main text.

A more detailed justification is provided in the Supplementary Information, based on three key ideas. First, any pattern (provided it is symmetric with respect to the initial spike) can be described as an arrangement of peaks with varying heights and spatial positions along a one-dimensional domain. Second, there exists a simple gene network—the diamond network—

that, through parameter tuning, can produce two peaks of arbitrary height and symmetric position relative to the initial spike. Third, by placing multiple diamond networks positively upstream of a common gene product, that gene product can express peaks at each location where the upstream diamond networks induce them. Under mild additional conditions, this mechanism allows the formation of essentially any symmetric pattern. These mild conditions, along with a detailed analysis of the diamond network's ability to generate peaks with controllable height and position, are discussed in the Supplementary Information.

> *"In 2D there are no peaks but concentric rings of high gene product concentration centered around the spike, while in 3D there are concentric spherical shells." (Main text l. 447ff).*

This result pertains specifically to pattern transformations arising from spike initial patterns. As defined in the text, spike initial patterns are radially symmetric. Since diffusion preserves radial symmetry, pattern transformations from spike initial patterns in two or three dimensions reduce to effectively one-dimensional transformations along each radial direction. In this framework, each pair of concentration peaks symmetric with respect to the spike in one dimension corresponds to a ring surrounding the spike in two dimensions, and each ring in two dimensions becomes a hollow spherical shell around the spike in three dimensions.

We agree that including a brief section in the Supplementary Information to clarify these subtleties would be helpful for readers to better understand the generalization of certain patterns to higher dimensions.

> *(4) The study identifies one-signal networks and examines how combinations of these structures can give rise to minimal pattern-forming subnetworks. However, the analysis of the combinations of these minimal pattern-forming subnetworks remains relatively brief, and the manuscript does not explore how the results might change if the subnetworks were combined in upstream and downstream configurations. In our view, it is not evident that all possible gene regulatory networks can be fully characterized by these categories, nor that the resulting patterns can be reliably predicted. Rather, the approach appears more suited to identifying which known subnetworks are present within a larger network, without necessarily capturing the full dynamics of more complex configurations.*

We acknowledge that our explanation regarding the combination of sub-networks was relatively brief, and we intend to address this in a revised version. Our argument that combining sub-networks does not produce qualitatively new types of pattern transformations -beyond those already described- is based on the dispersion relation. Although this relation was only detailed in the Supplementary Information, it is central to our argument and will therefore be moved to the main text. Below, we provide an outline of this argument:

Our study identifies two distinct behaviors of the principal branch of the dispersion relation at large wavenumbers. Based on this, gene networks capable of pattern formation can be classified into two categories: networks of the first kind, where the real part of the principal branch diverges to infinity as the wavenumber increases; and networks of the second kind, where the real part of the principal branch converges to a positive finite value for large wavenumbers. Naturally this argument applies to any gene network irrespectively of which, or how many, sub-networks are used to built it.

Any gene regulatory network capable of pattern formation falls into one of these two categories. We identified that networks of the first kind contain at least one Turing sub-network, whereas networks of the second kind include either an H sub-network or a noise-amplifying sub-network. In this way, the primary objective of our study -namely, achieving a

topological classification of gene regulatory networks capable of pattern formation- is fulfilled. It is important to note that while the dispersion relation provides broad information about the possible resulting patterns a gene network topology can produce (e.g., periodic versus noisy), it does not specify the exact patterns that emerge for each particular set of parameter values.

Finally, regarding the shape of the resulting patterns, Figure S10 in the Supplementary Information exemplifies the notion that the behavior of combined networks can be understood as a combination of the individual behaviors of each constituent sub-network (note that the contribution of each type of sub-network in the resulting pattern is readily distinguishable). Consequently, we focus our detailed analysis on the patterning properties of the fundamental classes.

> *(6) The manuscript lacks a clear and detailed explanation of the underlying model and its assumptions. In particular, it is not well-defined what constitutes a "cell" in the context of the model, nor is it justified why spatial features of cells -such as their size or boundaries- can be neglected. Furthermore, the concept of the extracellular space in the one-dimensional model remains ambiguous, making it unclear which gene products are assumed to diffuse.*

The size of cells is ignored in our model because we assume that they are small enough with respect to the total size of the domain that the space continuous reaction-diffusion equation (equation (1) in the main text) holds. Conceptually, one could understand cells in our model each of the pieces in an even partition of the domain into small subdomains surrounding each position x. This is anyway the standard procedure in most models of pattern formation by reaction-diffusion in embryonic development.

For extracellular signals, we assume that $g(t,x)$ corresponds to the concentration of the signal in the extracellular space surrounding the cell located at position x. The extracellular space is any fluid medium for which Fick Laws apply and, therfore, the Fickian diffusion term in equation (1) is valid.

For intracellular gene products, we assume that $g(t,x)$ corresponds to the concentration of such gene product within the cell at position x (if the gene product in hand is a transcription factor, for example), or on its surface (if it is a membrane-bound receptor). When collapsed in the continuous equations there is not such difference between being strictly within the cell or on its boundary. The only important fact is that these gene products cannot diffuse.

Regarding cell boundaries, let us consider an extracellular signal s that regulates a transcriptor factor i within cells (in our model, i is an intracellular gene product). Such regulation shall be mediated by a membrane-bound receptor, which corresponds to intracellular gene product j. In terms of the gene regulatory network this is $s \to j \to i$. Cell boundary effects mentioned by the reviewer should be encapsulated in the specific functional form of the regulation function $\boldsymbol{f}(\boldsymbol{g})$, but they have no effect in the actual topology of the network. Consequently, they are out of the scope of this study: as we mentioned before, considering different non-linear terms for $\boldsymbol{f}(\boldsymbol{g})$ will affect the parameter range for which a gene network is capable of producing non-trivial pattern transformations, but not their overall ability to produce non-trivial pattern transformations (i.e., the existence of at least one choice of model parameters for which such transformations take place).

Finally, we would like to once again express our sincere gratitude to all reviewers for their insightful and constructive feedback. We are confident that the thorough peer review process will significantly enhance both the clarity and depth of our work. We greatly value the detailed comments provided and will carefully incorporate them in the preparation of a revised manuscript, which we intend to submit in the coming months.

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