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

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eLife Assessment

The study presents a **valuable** conceptual framework by classifying pattern-forming gene subnetworks into three established categories. However, the supporting evidence remains **incomplete**, as the mathematical generalizations rely on simplified 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 address two question: 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 combine logical arguments and mathematical proofs to show that, despite the large amount of formally possible gene network topologies, all gene network topologies capable of pattern formation fall into only three fundamental classes and their combinations. Additionally, 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. This zoo includes the complete gene networks that, to the best of our knowledge, have been experimentally reported to lead to pattern formation as well as other gene networks that have not yet been found experimentally.

Significance Statement

Pattern formation is a central problem in developmental biology, yet the principles linking gene regulatory network topology to spatial patterning in multicellular systems are not fully understood. In this work, we identify logical and mathematical principles that determine which gene regulatory network topologies can give rise to spatial pattern formation through extracellular signaling. We further show that these topologies can be systematically classified into a small number of fundamental classes associated with distinct dynamical behaviors. Despite the vast number of *a priori* possible gene network configurations, the gene networks capable of stationary pattern formation fall into just three fundamental topological classes and their combinations. Gene networks within each class implement a common patterning logic and consequently generate analogous pattern transformations, revealing a unifying organizational framework underlying biological pattern formation.

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, 2023). 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 removed 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). In this sense, pattern formation in development does not usually start from a spatially homogeneous initial condition but from an initial condition that has some simple spatial heterogeneities. In that sense, we use the term pattern transformation instead of pattern formation (Salazar-Ciudad *et al*., 2003).

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 distribution of cell types in space or a specific distribution of gene product concentrations over space. For example, the earliest such patterns would be the zygote and the latest the adult. In most animals, early 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 transcription factors that lead to the expression of further genes, i.e., the transcription and translation of genes and the eventual synthesis of their gene products (Gilbert and Barresi, 2023). Other gene products are secreted and diffuse in the extracellular space and bind to specific receptors in distant cells (Gilbert and Barresi, 2023). 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).

Cells can respond to extracellular 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).

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. 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 in developmental biology is how pattern transformations occur. In animals, pattern transformation involves gene networks, signaling by extracellular signals and the regulation of cell behaviors (e.g. cell division, cell contraction, cell adhesion) and the mechanical properties of cells and tissues (Salazar-Ciudad, et al., 2003). As a preliminary step to understand pattern transformation by these processes, we restrict ourselves to the pattern transformations occurring through gene networks and extracellular signaling alone (not including membrane-tethered signals or mechano-transduction). We ask:

1. Which are the gene networks topologies that can lead to pattern transformation?
2. Can we identify all these topologies and classify them into classes leading to similar pattern transformations?

Among pattern transformations we are only interested in those that are non-trivial. By non-trivial pattern transformations we mean transformations in which:

P1. The resulting pattern is stationary and heterogeneous in space.

P2. There is at least one gene product that, in the resulting pattern, has a new spatial distribution of concentration maxima and minima over space (i.e. critical points of the concentration distribution over space, see S1 of the SI for details). By new we mean that no gene product had this spatial distribution of concentration maxima and minima in the initial pattern (see Fig.1). Thus, non-trivial pattern transformations include the emergence of new maxima or minima, the disappearance of existing ones, or their spatial displacement from one location to another. This excludes the simple widening of already existing concentration maxima, since, in this case, the position of the maxima does not change (see S1 in SI for further details). We only consider resulting developmental patterns that are stable in time. We say that a gene product has been patterned if the spatial distribution of its maxima and minima is new (i.e. not present in any gene product in the initial pattern).

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 these studies are restricted to networks of three gene products (Cotterel and Sharpe, 2010; Zheng *et al*., 2016) while others are restricted to studying 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 questions considered here and identified two classes of gene network topologies capable of pattern transformation (Salazar-Ciudad, 2000). However, this previous study did not show why the identified topology classes are the only possible ones. In addition, due to its purely numerical approach, this previous study failed to identify one class of gene network topologies capable of non-trivial pattern formation. Here we take a more general analytical mathematical approach to identify and characterize all possible classes, regardless of the number of gene products they entail.

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 basic cellular functions, etc.). However, the topic of this article is fundamentally different from those. Here we are not dealing with gene networks within a single cell, but with systems of cells with identical gene networks that are coupled through extracellular signaling. Specifically, we are interested in those genes networks leading to non-trivial pattern transformation (which is an inherently spatial question).

In this study, we consider three broad types of initial patterns (i.e., initial conditions): homogeneous-with-noise initial patterns, spike initial patterns and combined spike-homogeneous initial patterns. In a homogeneous-with-noise initial pattern, some gene products have zero concentration everywhere while others have the same non-zero concentration everywhere except for some small random fluctuations due to noise at the molecular level. In a spike initial pattern, the concentration of all gene products is zero everywhere except in a region (i.e. the spike region made of a small number of cells) where some gene products have the same non-zero concentration. In the combined spike-homogeneous initial pattern, some gene products have zero concentration everywhere while others have the same non-zero concentration everywhere except in the spike, where the concentration is larger (see Fig. 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 how gene product interactions can be wired into networks with different topologies to transform these initial patterns into other (i.e. resulting patterns) non-trivial ones.

It is worth noting that these three basic initial patterns correspond to spatially discontinuous functions: in homogeneous-with-noise initial patterns, white noise is discontinuous by definition; in spike and combined spike-homogeneous initial patterns, there is a concentration discontinuity

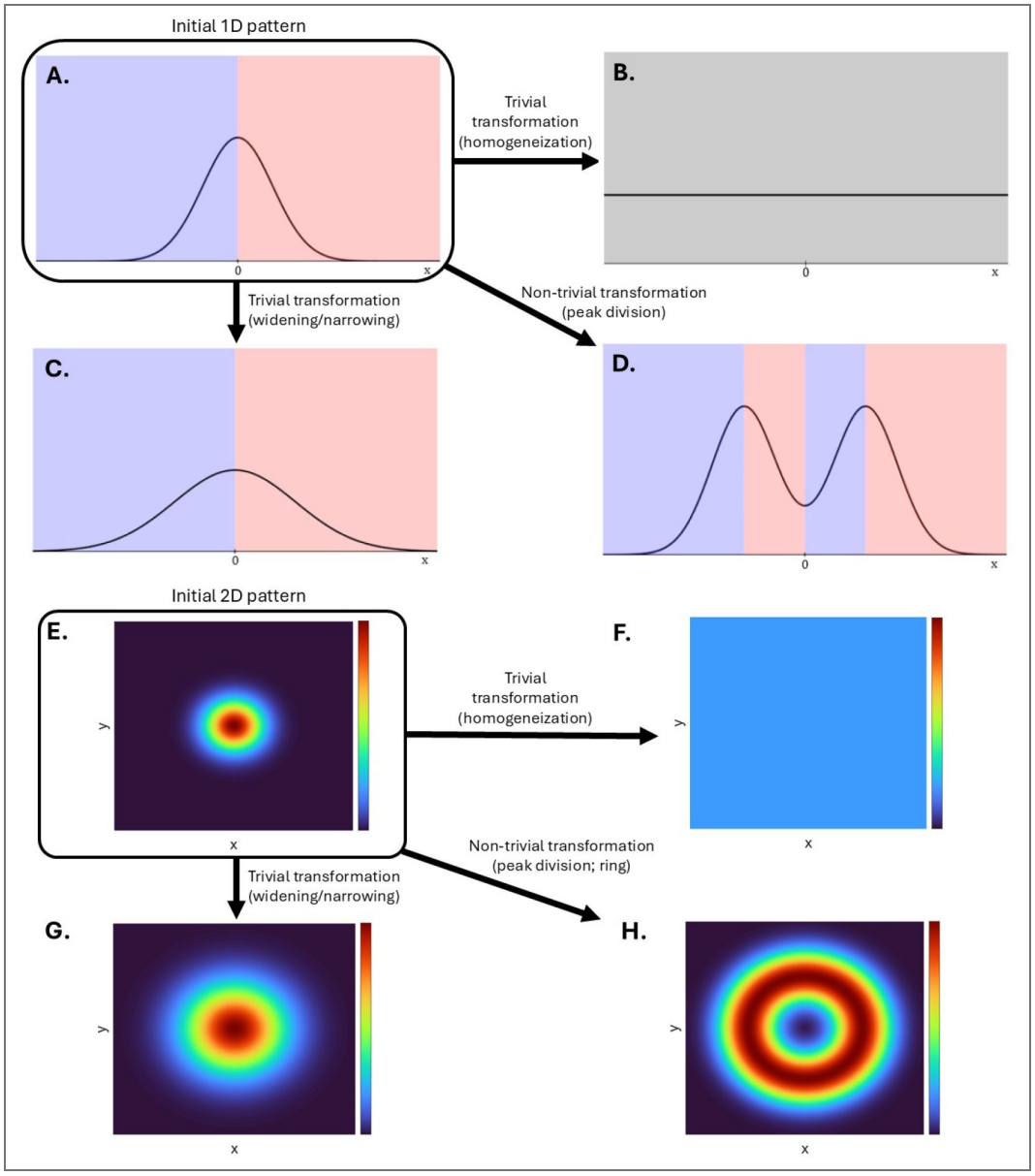


Figure 1. Pattern transformations.

The figure shows examples of pattern transformations in 1D **(A-D)** and in 2D **(E-H)**. Panels represent different patterns (i.e., different spatial distributions of some gene product concentration $g(t,\boldsymbol{x})$). (**A**) and **(E)** are the initial patterns being transformed into the resulting patterns in **(B-D)** and **(F-H)**. The x-axis is cell position along a 1D array of cells and the y-axis is the concentration of a gene product. The transformation from **(A)** (resp., **(E)**) into **(B)** (resp. **(F)**) is trivial because the resulting pattern is homogeneous. The transformation from **(A)** (resp., **(E)**) to **(C)** (resp., **(G)**) is trivial because the concentration maximum is at the same spatial position in both patterns. The transformation from **(A)** (resp., **(E)**) to **(D)** (resp., **(H)**) is non-trivial because the resulting pattern is heterogeneous and the pattern in **(D)** has maxima that were not present in **(A)**. The blue, grey and pink fillings in **(A-D)** correspond to the sign of the derivative and are meant to highlight the maxima and minima. In **(E-H)** the colors represent gene product concentration.

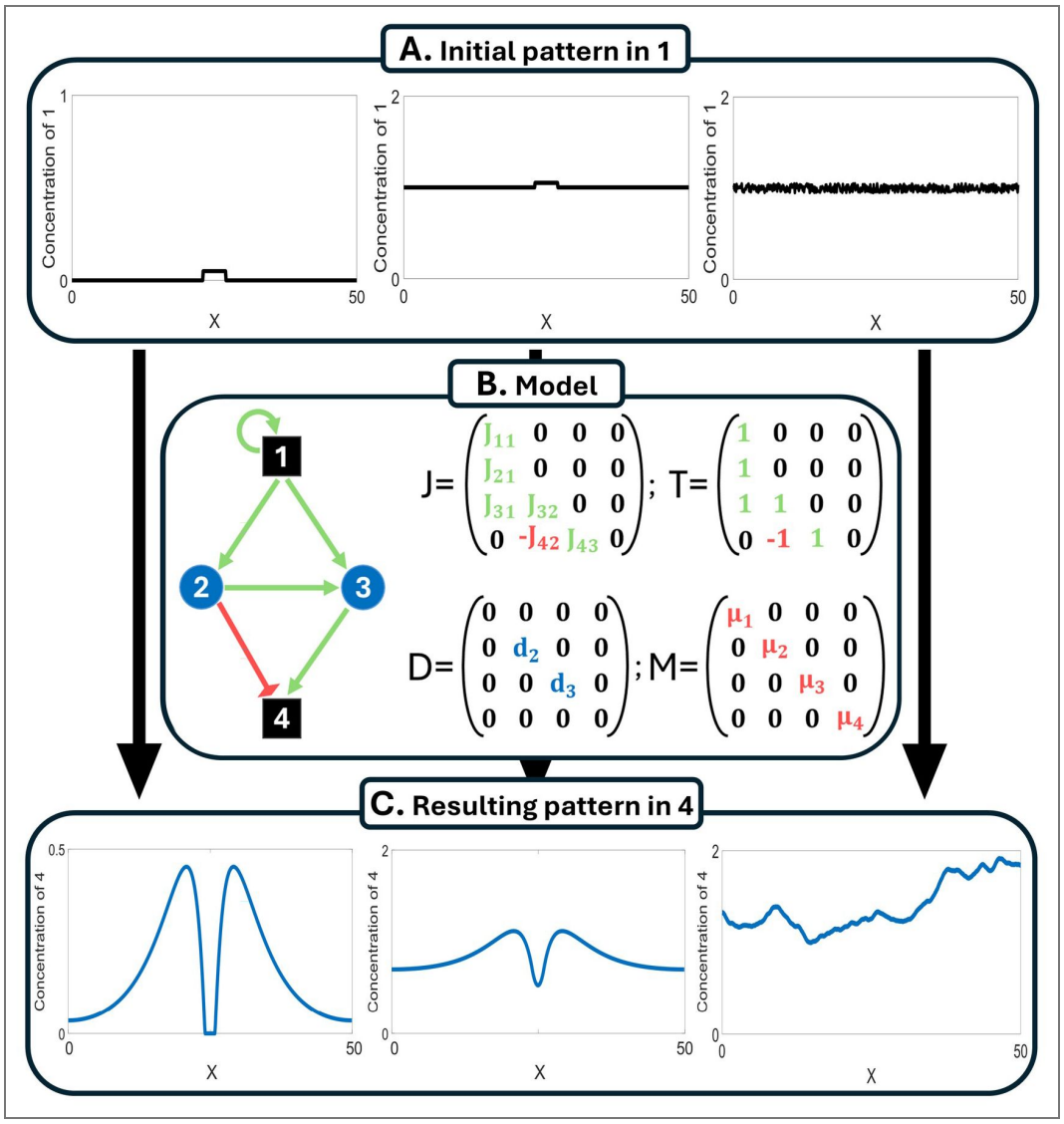


Figure 2. The model transforms initial patterns (A), into resulting patterns (C), through a set of equations implementing gene networks with extracellular signaling (B).

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

between cells on the edge of the spike and nearby cells outside the spike. However, once extracellular signal diffusion begins, these sharp boundaries are smoothed into differentiable gradients, where critical points can be properly defined (e.g., at the center of the initial spike).

Methods: The model

This article considers a set of N_c cells, each with an identical gene network of N_g gene products. For the purpose of clarity we explain our results as if cells have simple spatial arrangements (e.g., a line in 1D, a square lattice in 2D, etc.) and we later discuss them for more complex arrangements of cells in space. We refer to such spatial arrangement as the domain of our system. We consider two types of gene products: intracellular gene products and extracellular signals. The extracellular signals are gene products that are secreted by cells and diffuse in the extracellular space while intracellular gene products are gene products that are not. Even though we only use the general term gene product, our model applies, in principle, to any molecule whose concentration changes as a consequence of the concentration of other molecules in cells.

We consider that extracellular signals diffuse in the extracellular space immediately apical to cells and, therefore, that diffusion occurs in a space with the same spatial dimension than the arrangement of cells. In a 2D lattice of cells, for example, we consider that diffusion occurs in a 2D extracellular space immediately apical to cells. We do not consider any spatial information at scales smaller than a cell (i.e. cells are considered as points). Thus, we treat the extracellular space as an arrangement of contiguous regions, one per cell, and a single concentration value per gene product and region (i.e. concentration variations within each region are not considered, see Fig.3). We consider systems made of many small cells in which diffusion can be approximated by classical continuous equations.

For intracellular gene products, $g_i(t, x)$ denotes the concentration of gene product I inside cell at position x at time $t \geq 0$. For extracellular signals, $g_i(t, x)$ denotes the concentration of gene product i (i.e., signal) in the region of the extracellular space immediately apical to the cell at position x.

The dynamics of gene product concentrations in each cell in our model obey the following system of N_g reaction-diffusion equations (Murray 2002),

$$\partial_t \boldsymbol{g}(t,\boldsymbol{x}) = \boldsymbol{f}(\boldsymbol{g}(t,\boldsymbol{x})) - \boldsymbol{M}\boldsymbol{g}^{m}(t,\boldsymbol{x}) + \boldsymbol{D}\nabla_x^2 \boldsymbol{g}(t,\boldsymbol{x}),\ \text{for all } t>0 \text{ and all } \boldsymbol{x} \tag{1}$$

With initial pattern (i.e. initial condition),

$$\boldsymbol{g}(0,\boldsymbol{x}) = \boldsymbol{g}_0(\boldsymbol{x}),\ \text{for all } \boldsymbol{x} \tag{2}$$

And boundary conditions,

$$\hat{\boldsymbol{n}}\nabla_x \boldsymbol{g}\,(t,\boldsymbol{x}_b) = 0,\ \text{for all } t>0 \text{ and all boundary } \boldsymbol{x}_b, \tag{3}$$

where ∂_t is the time derivative ; $\boldsymbol{x}$ is space in (n=1,2,3 dimensions) ; $\boldsymbol{g}(t,\boldsymbol{x}) = \left(g_1(t,\boldsymbol{x}),\ldots,g_{N_g}(t,\boldsymbol{x})\right) \in \mathbb{R}_{\geq 0}^{N_g}$ is the vector of gene product concentrations (from gene 1 to N_g) at the cell at time t and position $\mathbf{x}$. $\boldsymbol{g}^{\boldsymbol{m}}(t,\boldsymbol{x}) = \left(g_1^{m_1}(t,\boldsymbol{x}),\ldots,g_{N_g}^{m_{N_g}}(t,\boldsymbol{x})\right) \in \mathbb{R}_{\geq 0}^{N_g}$ is a vector of powers of the gene product concentrations and $\boldsymbol{m}$ is a vector of positive integer exponents; $\boldsymbol{f} = \left(f_1,\ldots,f_{N_g}\right) : \mathbb{R}^{N_g} \to \mathbb{R}^{N_g}$ governs the interactions between gene products and determines how much of each gene product i is being synthesized in a given instant of time. $\boldsymbol{f}$ is, thus, a function that takes a vector of concentrations as input and returns another vector of concentrations as output ; ∇^2 denotes the Laplace operator (i.e., $\partial_{xx}^2 \boldsymbol{g}(t,\boldsymbol{x})$ in $\left(\partial_{xx}^2 + \partial_{yy}^2\right)\boldsymbol{g}(t,\boldsymbol{x})$ in 2D; in $\left(\partial_x^2 + \partial_y^2 + \partial_z^2\right)\boldsymbol{g}(t,\boldsymbol{x})$ in 3D). $\boldsymbol{D} = \operatorname{diag}\left(d_1,\ldots,d_{N_g}\right)$ is an $N_g \times N_g$ -diagonal matrix with real positive diffusion coefficients for each gene product along its diagonal. By definition extracellular signals have $d_i > 0$ while for intracellular gene products $d_i = 0$. Similarly, $\boldsymbol{M} = \operatorname{diag}\left(\mu_1,\ldots,\mu_{N_g}\right)$ is a $N_g \times N_g$-diagonal matrix with the real degradation coefficients for each gene product along its diagonal, $\mu_i \geq 0$. The degradation coefficient of a gene product is the default rate at which it would be degraded by proteases if no other factors affect its degradation. Notice that equation (1) has N_g components and applies to each cell.

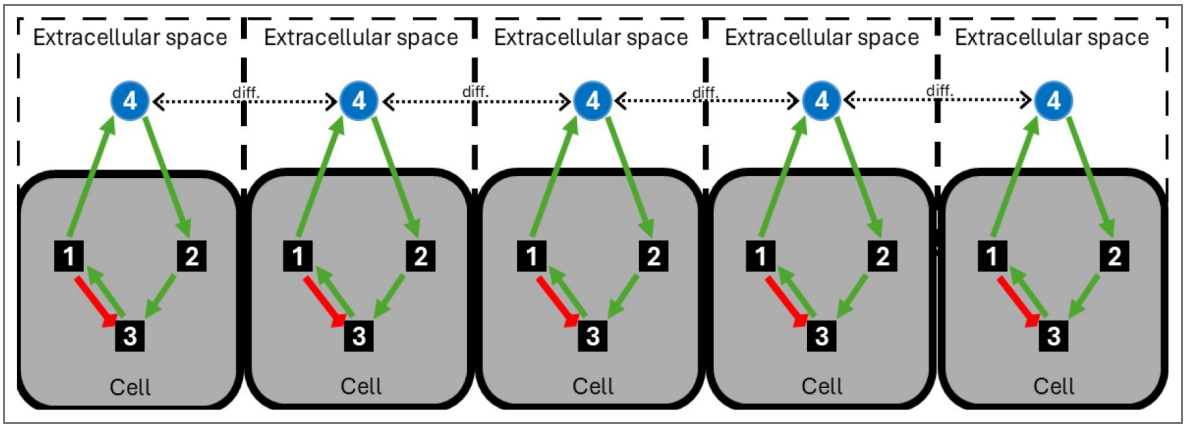


Figure 3. **Schema of the elements and spatial relationships in the model.**

Gray squares are cells. Each white square represents the extracellular space just apical of each cell. Small black squares represent intracellular gene products. Blue circles represent extracellular diffusible signals. Dashed lines represent the diffusion of the extracellular signals over extracellular space. Full green lines represent positive regulation between gene products. Red full lines represent inhibitory regulation between gene products.

For the cases of extracellular signals, i.e. their concentration in a cell can affect that in distant cells through the diffusion term (last term in (1)).

In (2), $\boldsymbol{g_0}(x)$ represents the initial pattern (i.e. initial spatial condition). In (3), $\hat{n}\nabla_x \boldsymbol{g}(t, x_b) = 0$ are the boundary conditions of the system (zero flux boundary conditions). ∇ denotes the nabla operator (i.e., $\partial_x \boldsymbol{g}(t, \boldsymbol{x})$ in 1D; $(\partial_x + \partial_y)\, \boldsymbol{g}(t, \boldsymbol{x})$ in 2D; and $(\partial_x + \partial_y + \partial_z)\, \boldsymbol{g}(t, \boldsymbol{x})$ in 3D.); and $\hat{n}$ is a unit vector normal to the boundary of the cell arrangement.

The last term in (1) is Fick's second law of diffusion (Fick, 1855) describing the contribution of extracellular diffusion to changes in the concentration of extracellular signals. Our model does not consider membrane-bound signals (see Salazar-Ciudad *et al*., 2000 for a similar study that does).

The first two terms in system (1) can be understood as a reaction term. $\boldsymbol{f}$ defines a directed graph via its Jacobian matrix $\boldsymbol{J}$ (i.e., the matrix of first derivatives of $\boldsymbol{f}$ with respect to each gene product concentration), where each node stands for a given gene product, and each edge describes an interaction between gene products (Fig.2B).

Following the standard terminology in molecular biology, we say that a gene is being expressed in a cell at a give time if its gene product is being produced by that cell (i.e. the first term of (1) is larger than zero for that cell at that time). Notice that, because of the diffusion term, extracellular signals can have positive non-zero concentration around cells that are not expressing them.

We say that gene product j directly regulates gene product k if the corresponding element of the Jacobian matrix of $\boldsymbol{f}$ 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). Similarly, we say that k is downstream of j if there exists a chain of regulations between gene products going from j to k. Correspondingly, j is said to be upstream from k. Notice that a gene product can be both upstream and downstream of a set of gene products, thus forming a regulatory loop. We call extracellular loops those regulatory loops in which at least one gene product is an extracellular signal.

Our results are only valid for functions $\boldsymbol{f}$ and reactions terms (i.e., $\boldsymbol{f}(\boldsymbol{g}) - \boldsymbol{Mg^m}$) that satisfy some specific requirements. These are very broad biological requirements that are likely to be fulfilled by many developmental gene networks.

Requirements on the reaction term and f

R1

$\boldsymbol{f}$ is continuous and continuously differentiable (at least locally), so that its Jacobian matrix can be properly defined. Within each cell, we assume that the number of copies of each gene product and its rate of production are large enough for its concentration to be treated as continuous, as in many other models of pattern formation (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). Notice that the degradation term in (1) is also continuous and continuously differentiable, $-\boldsymbol{Mg^m}(t, \boldsymbol{x})$.

R2

$\boldsymbol{f}$ is such that $\boldsymbol{f}(\boldsymbol{g}(t, \boldsymbol{x})) - \boldsymbol{Mg^m}(t, \boldsymbol{x})$ is non-linear in at least one of its components. In other words, at least for one gene product, the dependence of concentration on that of other gene products (or its own) is not linear. This is because it is well known that no non-trivial pattern transformation is possible in purely linear systems (Kondepudi & Prigogine, 2014; Murray, 2002).

R3

$\boldsymbol{f}$ is an explicit function of $\boldsymbol{g}$, but not an explicit function of time or any time derivative of $\boldsymbol{g}$. 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*** is always non-negative and bounded. This is because ***g*** is a vector of concentrations and concentrations cannot be negative. Similarly, cells are finite and, hence, cannot produce an infinite amount of gene products. Thus, ***f*** should be such that gene product concentrations never go to infinity.

R5

f is monotonously increasing or decreasing with respect to each gene product concentration. In other words, the sign of gene products interactions cannot change with the concentration of the interacting gene products (when everything else is kept constant). Thus, if a gene product *k* activates another gene product *j*, then *k* is an activator of *j*, even if its concentration changes. The same applies to inhibitory interactions (i.e. the sign of the regulation of *k* by *j* does not change with the concentration of *j*). In other words, the partial derivative of ***f*** with respect to each gene product *j*, should have the same sign for any value of g_j,

$$J_{kj} = \frac{\partial f_k}{\partial g_j} \geq 0(\text{ or } \leq 0),\ \text{for any k, j and } g_j \tag{4}$$

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 for the evaluation of J_{kj}. There are some known examples of particular gene product interactions that do not fulfill this requirement (Nelson *et al*., 2021) but, in general, the sign of the regulation of a gene product by another does not change in any complex way with the concentration of either gene product. Even in the reported cases where this happens, sign changes only occur once or twice (Nelson *et al*., 2021). Requirement R5 is simply a way to consider that pattern formation does not just arise from very simple gene networks with very complex interactions between each pair of genes (i.e. changes in the sign of the regulation depending on the concentration of the regulator) but from networks of relatively simple interactions between gene products.

Through this article when we refer to the "the parameters" we mean the parameters described in (1) (i.e. its parameters, ***M, D*** and ***J***) and to any additional parameters a specific ***f*** may have. We consider that these parameters do not vary over time or space.

In this article 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 entry in the Jacobian matrix (i.e., $T = sgn(J)$) (see Fig.2B). The topology of a given gene network is then a description of which gene products interact with which gene products and the sign of that interaction. In contrast, from now on, we will reserve the term gene network for a gene network topology with a specific ***f*** and all its parameter values so that a specific resulting pattern can arise from a specific initial pattern (i.e. a full implementation of equations (1)-(3) for specific ***f*** and specific values of the parameters). In this article a topological class is a set of topologies that fulfill some requirement definable at the level of topology, e.g. they have at least one positive loop. Studying gene network topology is important because for most developmental systems we only have information about *T*, and not so much about ***f*** (Gilbert and Barresi, 2023).

The results are organized into a set of logical arguments (for the least evident of them, we provide mathematical proofs in the Supporting Information). First we present some trivial requirements that the topology of all pattern-transforming gene networks need to fulfill. Secondly, we explain why all gene networks leading to non-trivial pattern transformations can be classified into three topological classes, and their combinations. Thirdly, we explain how the topological class to which a gene network belongs determines which non-trivial pattern transformations it can produce and which ones it cannot produce. In other words, belonging to one of these three classes is a necessary, but not sufficient, condition for a gene network to be able to lead to non-trivial pattern transformations. Among the types of pattern transformations possible from its topological class, a given gene network would produce one or another depending on the exact form of ***f*** and the value of the parameters. However, the topological class to which a gene network belongs indicates which non-trivial pattern transformations it cannot produce, regardless of its parameters and ***f***.

Results

Any network with more than two gene products can be partitioned into a set of subnetworks but, as we will see, partitioning gene networks based on extracellular signals is especially useful. In this article, we call a *signal subnetwork* to the set of interactions between all gene products downstream of an extracellular signal (including the signal itself). Signal subnetworks, or from now on simply subnetworks, can partially overlap since a gene product can be downstream of several extracellular signals (see Fig.4). An extracellular signal can be upstream of other extracellular signals and, thus, a signal subnetwork can include other signal subnetworks (see Fig.4).

Basic requirements on gene networks capable of pattern transformation

In this section we present some simple requirements on the topology of any gene network capable of pattern transformation. These are rather trivial but introducing them here facilitates explaining later results.

I1

Any gene network capable of non-trivial pattern transformation must include at least one signal subnetwork whose extracellular signal is either present in the initial pattern or positively downstream of some gene product that is. By 'present in the initial pattern', we mean that the corresponding gene product has a non-zero initial concentration somewhere in the domain (i.e., its gene is expressed in the initial pattern). Since we do not consider cell movement, mechano-transduction, and membrane-tethered signaling, non-trivial pattern transformations can only occur through the secretion of extracellular signals, which necessarily requires the activation of at least one signal subnetwork.

I2

All gene products are being degraded at all times (second term in (1)). Thus, for a gene product to be present in the resulting pattern, it should receive positive regulation from some gene products. This ultimately requires that all the gene products present in the resulting pattern are either within a self-activatory loop or downstream of it (otherwise, their concentration will decay to zero over time). This broad case includes genes that are constitutively expressed, meaning that they are always produced by cells regardless of other gene products (this can be viewed as these genes being within a self-activatory loop of their own or downstream of one).

Considering I1 and I2, it follows that all gene networks capable of non-trivial pattern transformation should contain a positive regulatory loop that is upstream of the patterned gene products and downstream of gene products present in the initial pattern. These loops can be extracellular or intracellular (i.e., include extracellular signals or not).

Gene network classification

In this section we explain that all conceivable signal subnetworks can be classified into just three classes based on how cells respond to extracellular signals in terms of secreting, or not, the same extracellular signals (see Fig.4). Let A be the most upstream extracellular signal of a given signal subnetwork. Then, there are trivially only two options, either A is downstream of itself (directly or indirectly), or it is not. In the latter case, we say that the subnetwork is hierarchical, or class H, while in the former case we say that the subnetwork is emergent (Salazar-Ciudad *et al.*, 2000). Notice that this exhausts all possibilities: an extracellular signal is either downstream of itself or not, there are no other possibilities. Thus, all signal subnetworks can be classified as hierarchical, emergent or a combination of them (for subnetworks composed of other subnetworks).

The emergent class can be further divided into the L^+ class, if A is positively downstream (and upstream) of itself, or into the class L^-, if A is negatively downstream (and upstream) of itself. This gives three topological classes: H, L^- and L^+.

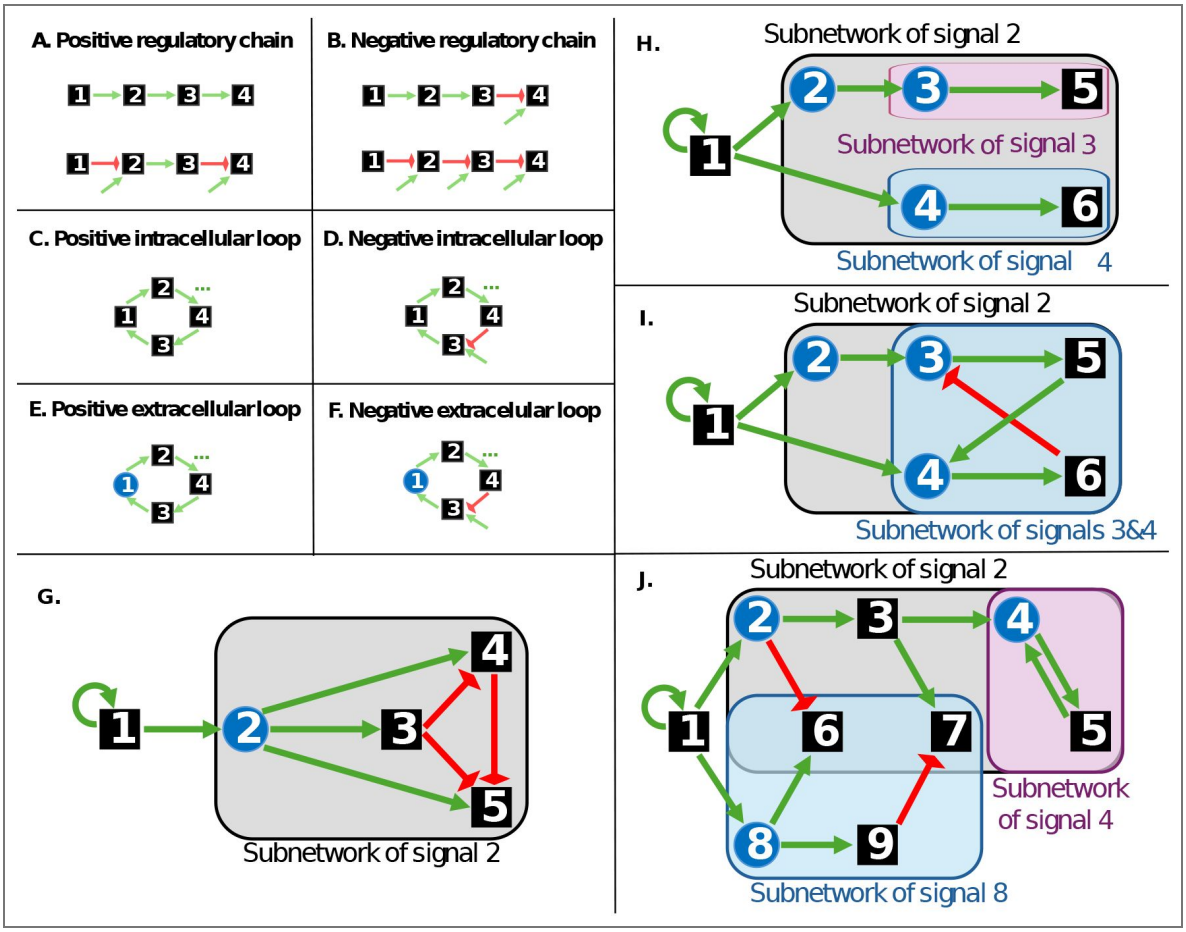


Figure 4. Definition of chains, loops, and signal subnetworks.

(A-D) Parts of intracellular gene networks **(E-F)** Schema of simple extracellular regulatory loops. **(G-J)** Examples of gene regulatory networks with their signal subnetworks depicted. Small black squares represent intracellular gene products, and blue circles represent extracellular diffusible signals. Green arrows denote positive regulation, and red blunt arrows denote inhibition. Dots (...) indicate any positive chain of gene product interactions. **(A)** A pPositive regulatory chain is a sequence of interactions in which each gene product positively regulates the next and is positively regulated by the previous one in the sequence. A sequence with an even number of inhibitory interactions is also a positive regulatory chain, provided that each inhibited gene product receives a positive input from elsewhere. **(B)** Negative regulatory chain: as in **(A)** but with an odd number of inhibitory interactions. **(C)** A positive intracellular loop is a positive regulatory chain whose first and last gene products coincide and in which all gene products are intracellular. **(D)** Negative intracellular loop: as in **(C)** but with an odd number of inhibitory interactions. **(E)** A positive extracellular loop is a positive regulatory loop in which at least one gene product is an extracellular signal. **(F)** Negative extracellular loop: as in **(E)** but with an odd number of inhibitory interactions. **(G)** An example network in which we have surrounded its signal subnetwork with a grey rectangle. **(H)** Example network with two signal subnetworks. **(I)** Example network with three extracellular signals; each signal subnetwork is enclosed by a rectangle. Note that since signal 3 and 4 are in a loop their signal subnetworks include the same gene products. **(J)** Another example network with its subnetworks indicated.

H subnetworks can contain regulatory loops as long as these do not include an extracellular signal (because in this case the loop would be extracellular, and the subnetwork, emergent). In fact, because of I2, any H network leading to pattern transformation must contain a positive intracellular loop (we label these as I^+).

A gene network topology can be composed of several signal subnetworks. We classify whole gene networks topologies in the same way as their composing subnetworks. For example, a gene network topology with only H subnetworks is an H gene network, while a gene network topology with only L^+ subnetworks is an L^+ network. Composite gene networks topologies are labeled according to their composing subnetworks, independently of the number of subnetworks of each class they contain (see Fig.5). For example, an H L^+ gene network can contain one, or more, of H subnetworks, and one, or more, L^+ subnetworks.

Our classification into classes H, L^+ and L^- exhausts all possible topologies for gene networks. Within each topological class, however, there are gene networks that can lead to non-trivial pattern transformations and gene networks that cannot (e.g. depending on the parameters and the exact $\boldsymbol{f}$). In the rest of this article we first identify further topological requirements that gene networks of each class must satisfy in order to lead to non-trivial pattern transformations. Then, we study the types of resulting patterns possible from each topological class. These types are described in very broad terms. In other words, we do not specify which exact resulting patterns arise from each gene network but some general features and commonalities among the resulting patterns possible from the gene networks within each class. We do the same for the gene networks combining different classes of subnetworks.

Linear stability analysis

In this section, we introduce the mathematical apparatus that allows us to link the topology of a gene network to its ability to produce non-trivial pattern transformation: the dispersion relation. We will treat each initial pattern as a perturbation of an otherwise spatially homogeneous steady state, and we will study the conditions under which such perturbation grows in time. If initial perturbations do not grow, one recovers the original homogeneous steady state and, thus, there is no pattern transformation. Even when these perturbations grow, one may recover a different homogeneous steady state as a resulting pattern, i.e. no non-trivial pattern transformation either. Thus, instability against perturbations is a necessary but no sufficient condition for pattern transformation.

To analyze whether a perturbation will grow we will use the standard linear stability analysis of the dynamical system defined by our model equations (1)-(3). In this study we focus in those $\boldsymbol{f}$ functions for which equation (1) to (3) admit a steady state homogeneous solution $\boldsymbol{g}(t, \boldsymbol{x}) \equiv \boldsymbol{g}^*$ (i.e. a homogeneous steady state) given by $\boldsymbol{f}(\boldsymbol{g}^*) - \boldsymbol{M}(\boldsymbol{g}^*)^{\boldsymbol{m}} = 0$. Hence, let $\tilde{\boldsymbol{g}}(t, \boldsymbol{x}) = \boldsymbol{g}(t, \boldsymbol{x}) - \boldsymbol{g}^*$ denote a small concentration perturbation from the homogeneous steady state $\boldsymbol{g}^*$. Then, plugging $\tilde{\boldsymbol{g}}(t, \boldsymbol{x})$ back into (1)-(3) shows that the evolution of such perturbation in space and time is given by the linearized reaction-diffusion equations,

$$\begin{cases} \partial_t \tilde{\boldsymbol{g}}(t, \boldsymbol{x}) = \boldsymbol{R}(0)\tilde{\boldsymbol{g}}(t, \boldsymbol{x}) & \text{for all } t > 0 \text{ and all } \boldsymbol{x}; \\ \quad + \boldsymbol{D}\nabla^2 \tilde{\boldsymbol{g}}(t, \boldsymbol{x}), & \\ \tilde{\boldsymbol{g}}(0, \boldsymbol{x}) = \tilde{\boldsymbol{g}}_{\boldsymbol{0}}(\boldsymbol{x}), & \text{for all } \boldsymbol{x}; \\ \hat{\boldsymbol{n}} \nabla \tilde{\boldsymbol{g}}\,(t, \boldsymbol{x}_b) = 0, & \text{for all } t > 0 \text{ and all boundary } \boldsymbol{x}_b; \end{cases} \tag{5}$$

where $\boldsymbol{R}(0) = \boldsymbol{J}_f^* - \boldsymbol{m} \odot \boldsymbol{M}(\boldsymbol{g}^*)^{m-1}$ is the reaction-diffusion matrix in the absence of diffusion; and $\tilde{\boldsymbol{g}}_{\boldsymbol{0}}(\boldsymbol{x}) = \boldsymbol{g}_0(\boldsymbol{x}) - \boldsymbol{g}^*$ represents the initial perturbation. In the reaction diffusion matrix, $J_f^* = J_f\,(g^*)$ is the Jacobian matrix of $\boldsymbol{f}$ (i.e. the matrix of derivatives of $\boldsymbol{f}(\boldsymbol{g})$ with respect to each gene product) evaluated at the steady state $\boldsymbol{g}^*$, and $\boldsymbol{u} \odot \boldsymbol{v} = (u_1 v_1, \ldots, u_{N_q} v_{N_q})$ denotes component-by-component vector multiplication.

Each initial pattern can be described as a different small initial perturbation $\tilde{g}_0(x)$ around an otherwise homogeneous steady state $\boldsymbol{g}^*$. In homogeneous-with-noise initial patterns, $\boldsymbol{g}^* > 0$ and $\tilde{\boldsymbol{g}}_0(x)$ is small white noise. In spike initial patterns, $\boldsymbol{g}^* = 0$ and $\tilde{\boldsymbol{g}}_0(x) > 0$ only for positions $\boldsymbol{x}$ such

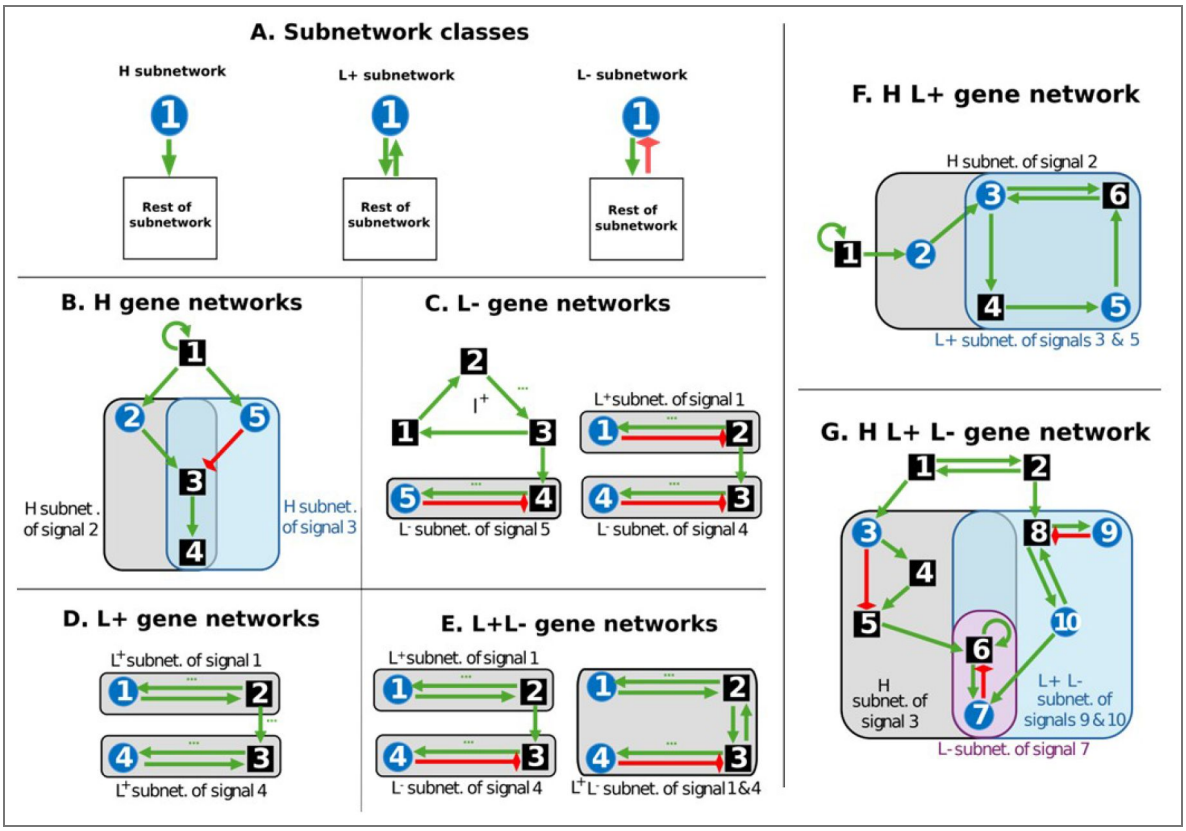


Figure 5. Subnetwork and network classification.

(A) Schema representing each class of subnetwork. In a H signal subnetworks, the extracellular signal is not downstream of itself. In a signal L^+ subnetworks, the extracellular signal is both upstream and downstream of itself, forming a positive extracellular loop (which may include any number of gene products). In a L^- signal subnetwork, the extracellular signal is negatively upstream and downstream of itself. The box labeled 'rest of subnetwork' represents any gene network provided that the most upstream extracellular signal is not negatively upstream of itself (i.e., no negative loop leads back to it). **(B) (B)** Example of a H gene network containing two H subnetworks. **(C)** Two examples of L^- gene networks, one with on L^- subnetwork (left) and one with two L^- subnetworks (right). **(D)** Example of a L^+ gene network with two L^+ subnetworks. **(E)** Two examples of L^+L^- gene networks. Each of them has a L^+ subnetwork and a L^- subnetwork. **(F)** Example of a H gene network. **(G)** Example of a gene network with a L^+ subnetwork, a L^- subnetwork and a H subnetwork. Colors of arrows and gene products as in figure 2 and 4.

that $||\boldsymbol{x}-\boldsymbol{x_c}|| < L$, where $\boldsymbol{x_c}$ is the center of the spike and L its width. In the combined spike-homogeneous initial pattern, $\boldsymbol{g^*}>0$ and, again, $\tilde{g}_0(x) > 0$ for positions $\boldsymbol{x}$ in the spike such that $||\boldsymbol{x}-\boldsymbol{x_c}|| < L$. In both cases, the initial concentration in the spike must be small enough for linearization (5) to hold (see S of the SI for the case of larger spikes).

Over time, the initial perturbation can either decay (i.e., $\tilde{\boldsymbol{g}}(t,\boldsymbol{x}) \to 0$ as $t \to \infty$) or grow away from the original homogeneous steady state. In the former case, we say that $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ is a stable perturbation; in the latter, that $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ is an unstable perturbation. Since we are interested in the gene networks that can lead to pattern transformations through extracellular signaling, we are interested in the gene networks in which perturbations are unstable when there is extracellular signal diffusion (i.e., when some elements of $\boldsymbol{D}$ are non-zero).

Solutions to (5) can be found using the spectral decomposition of the Laplacian ∇^2 with zero-flux boundary conditions (Murray, 2003). In this sense, let $W_{\boldsymbol{k}}(\boldsymbol{x})$ be a zero-flux eigenmode of the Laplacian operator in our domain, that is, a non-trivial solution (i.e., $W_{\boldsymbol{k}}(\boldsymbol{x})\neq 0$ for at least one $\boldsymbol{x}$) of the following equations,

$$\begin{cases} \nabla^2 W_k(\boldsymbol{x}) = -\boldsymbol{k}^2 W_k(\boldsymbol{x}), & \text{for all } \boldsymbol{x}, \\ \hat{\boldsymbol{n}} \nabla W_k(\boldsymbol{x}_b) = 0, & \text{for all boundary } \boldsymbol{x}_b, \end{cases} \tag{6}$$

for some $\boldsymbol{k}\in\mathbb{R}^n$, with n=1,2 or 3. We denote by $\sigma(\nabla^2)$ the countable set of all $\boldsymbol{k}\in\mathbb{R}^n$ for which equation (6) admits a non-trivial solution (Baker *et al.*, 2008), and we say that $\boldsymbol{k}\in\sigma(\nabla^2)$ are the wavenumbers of the corresponding eigenmode $W_{\boldsymbol{k}}(\boldsymbol{x})$. This is a slight abuse of notation based on the fact that, in simpler spatial domains (e.g., a 2D square), eigenmodes $W_{\boldsymbol{k}}(\boldsymbol{x})$ are trigonometric functions for which each entry in $\boldsymbol{k}$ counts the number of maxima and minima that $W_{\boldsymbol{k}}(\boldsymbol{x})$ displays along each spatial dimension.

Eigenmodes $W_{\boldsymbol{k}}(\boldsymbol{x})$ can be seen as a generalization of classical Fourier modes and hence, we can now consider the series expansion of $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ in terms of $W_{\boldsymbol{k}}(\boldsymbol{x})$, that is,

$$\tilde{\boldsymbol{g}}(t,\boldsymbol{x}) = \sum_{k\in\sigma(\nabla^2)} \hat{\boldsymbol{c}}_k(t) W_k(\boldsymbol{x}), \tag{7}$$

where each $\hat{\boldsymbol{c}}_k(t) \in \mathbb{R}^{N_g}$ is given by,

$$\hat{\boldsymbol{c}}_k(t) = \int \boldsymbol{g}(t,\boldsymbol{x}) W_k(\boldsymbol{x}) \mathrm{d}\boldsymbol{x}. \tag{8}$$

If we now plug series expansion (7) into system (5) and use the linearity of the equations to split the sum into terms for each $\boldsymbol{k}$, we get,

$$\partial_t \hat{\boldsymbol{c}}_k(t) W_k(\boldsymbol{x}) = \boldsymbol{R}(0)\hat{\boldsymbol{c}}_k(t) W_k(\boldsymbol{x}) + \boldsymbol{D}\hat{\boldsymbol{c}}_k(t)\nabla^2 W_k(\boldsymbol{x}); \tag{9}$$

and then, if we use (6) to solve the space derivatives, and eliminate alike terms from each side of the equations (i.e., $W_{\boldsymbol{k}}(\boldsymbol{x})$), we get that the time evolution of each $\hat{\boldsymbol{c}}_{\boldsymbol{k}}(t)$ is given by the equation,

$$\partial_t \hat{\boldsymbol{c}}_k(t) = \boldsymbol{R}\left(\boldsymbol{k}^2\right) \hat{\boldsymbol{c}}_k(t), \tag{10}$$

where $\boldsymbol{R}(\boldsymbol{k}^2)=\boldsymbol{R}(0)+\boldsymbol{k}^2\boldsymbol{D}=\boldsymbol{J_f}(\boldsymbol{g^*})-\boldsymbol{m}\odot\boldsymbol{M}(\boldsymbol{g^*})^{\boldsymbol{m-1}}$ is the so-called reaction-diffusion matrix. Solutions to equation (10) are given by,

$$\hat{\boldsymbol{c}}_k(t) = \exp\left(\boldsymbol{R}\left(\boldsymbol{k}^2\right) t\right) \hat{\boldsymbol{c}}_{\boldsymbol{k}}^{(0)}, \tag{11}$$

where $\exp(\boldsymbol{R}(\boldsymbol{k}^2)t)$ is a matrix exponential; and each $\hat{\boldsymbol{c}}_{\boldsymbol{k}}^{(0)} = \hat{\boldsymbol{c}}_{\boldsymbol{k}}(0) \in \mathbb{R}^{N_g}$ is computed as in (8), changing $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ by the corresponding initial perturbation $\tilde{\boldsymbol{g}}_0(x)$.

At this point, we can plug (11) back into (7) and thus, we get that solutions $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ to the linearized reaction-diffusion equations (5) can be written as a series expansion of the form,

$$\tilde{\boldsymbol{g}}(t,\boldsymbol{x}) = \sum_{\boldsymbol{k}\in\sigma(\nabla^2)} \exp\left(\boldsymbol{R}\left(\boldsymbol{k}^2\right)t\right)\hat{\boldsymbol{c}}_{\boldsymbol{k}}^{(0)} W_{\boldsymbol{k}}(\boldsymbol{x}). \tag{12}$$

However, for our linear stability analysis, we are only interested in the long-term behavior of (12), namely whether $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ grows over time or eventually decays back to 0. In this sense, the long-term behavior of $\exp(\boldsymbol{R}(\boldsymbol{k}^2)t)$ is dominated by the eigenvalue of $\boldsymbol{R}(\boldsymbol{k}^2)$ with the greatest real part and then, we can reduce expression (12) to,

$$\tilde{\boldsymbol{g}}(t,\boldsymbol{x}) \sim \sum_{\boldsymbol{k}\in\sigma(\nabla^2)} e^{\Lambda_k t}\boldsymbol{c}_{\boldsymbol{k}}^{(0)} W_{\boldsymbol{k}}(\boldsymbol{x}), \tag{13}$$

where $\Lambda_{\boldsymbol{k}}\in\mathbb{C}$ is the eigenvalue of $\boldsymbol{R}(\boldsymbol{k}^2)$ with the greatest real part, and $\boldsymbol{c}_k^{(0)} \in \mathbb{R}^{N_g}$ is the spectral projection of $\hat{\boldsymbol{c}}_{\boldsymbol{k}}^{(0)}$ onto the corresponding eigenspace with eigenvalue Λ_k.

The different eigenvalues of $\boldsymbol{R}(\boldsymbol{k}^2)$ vary with $\boldsymbol{k}$, and this variation is given by the so-called dispersion relation. By definition, the dispersion relation corresponds to the characteristic equation of the reaction-diffusion matrix $\boldsymbol{R}(\boldsymbol{k}^2)$ (Murray, 2003; Baker *et al.*, 2008), that is,

$$\det\left(\lambda\boldsymbol{I} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) = \det\left(\lambda\boldsymbol{I} - \boldsymbol{J}_f^* + \boldsymbol{m}\odot\boldsymbol{M}(\boldsymbol{g}^*)^{m-1}\right) = 0, \tag{14}$$

where $\boldsymbol{I}$ denotes the $N_g\times N_g$ identity matrix. Then, $\Lambda_{\boldsymbol{k}}\in\mathbb{C}$ in the exponent of (13) corresponds to the solution of (14) with the greatest real part for each given $\boldsymbol{k}$ (i.e., $\Lambda_{\boldsymbol{k}}=\lambda_i$ such that $\mathrm{Re}(\lambda_i)\geq\mathrm{Re}(\lambda_j)$ for any other solution λ_j of (14)). Accordingly, we will refer to $\Lambda_{\boldsymbol{k}}$ as the principal branch of the dispersion relation.

The principal branch in of the dispersion relation, (13), determines whether a perturbation is stable or unstable. If $\mathrm{Re}(\Lambda_{\boldsymbol{k}})<0$ for some $\boldsymbol{k}\in\sigma(\nabla^2)$, then $e^{\Lambda_k t}$ decays to 0 as $t\to\infty$ and we say that $\boldsymbol{k}$ (resp. $W_{\boldsymbol{k}}(\boldsymbol{x})$) is a stable wavenumber (resp., eigenmode). Conversely, if $\mathrm{Re}(\Lambda_{\boldsymbol{k}})>0$ for some $\boldsymbol{k}\in\sigma(\nabla^2)$, then $e^{\Lambda_k t}$ grows as $t\to\infty$ and we say that $\boldsymbol{k}$ is an unstable wavenumber (resp., eigenmode). Hence, perturbation $\tilde{\boldsymbol{g}}(t,\boldsymbol{x})$ is stable if all wavenumbers $\boldsymbol{k}\in\sigma(\nabla^2)$ are stable, and is unstable if there exists at least one unstable wavenumber $\boldsymbol{k}\in\sigma(\nabla^2)$ for which the principal branch has a positive real part. Note that, in accordance with requirement R4, the unbounded exponential growth of unstable eigenmodes in (13) will be halted, (13), eventually, by the higher order terms in (1) that we disregard in the linear approximation (6).

Each gene network has a $\boldsymbol{J}_f^*$, $\boldsymbol{M}$ and $\boldsymbol{D}$ matrices, and each such set of matrices defines a different reaction-diffusion matrix $\boldsymbol{R}(\boldsymbol{k}^2)$. This means that each gene network has and associated dispersion relation that is given by equation (14). In this sense, a gene network can only produce pattern transformations if its associated dispersion relation has at least one unstable wavenumber $\boldsymbol{k}\in\sigma(\nabla^2)$ for which $\mathrm{Re}(\Lambda_{\boldsymbol{k}})>0$. Moreover, each initial perturbation $\tilde{\boldsymbol{g}}_0(x)$ can be described as a series expansion in terms of wavemodes $W_{\boldsymbol{k}}(\boldsymbol{x})$ (e.g., a homogeneous-with-noise initial pattern will result in a series with many eigenmodes $W_{\boldsymbol{k}}(\boldsymbol{x})$ and many of them with large wavenumbers $\boldsymbol{k}$). In this sense, each gene network can be seen as leading, or not, to pattern transformation by specifically destabilizing some of these wavenumbers while stabilizing others. The later means that some perturbations, the ones with the destabilized wavenumbers, grow over time until some non-linearities in $\boldsymbol{f}$ (R2 and R4) may preclude their further growth. This means that the resulting

patterns, can be characterized, to a large extent, by these destabilized eigenmodes (Murray, 2003), even though some other wavenumbers may be destabilized by nonlinear effects (e.g., resonant eigenmodes (Castelino *et al*., 2020)).

The principal branch (i.e. $\Lambda_{\boldsymbol{k}}$) allows to classify all the pattern transformations arising from gene networks into two mutually exclusive types: those with a finite amount of unstable wavenumbers and those with an infinite amount of unstable wavenumbers.

We say that a gene network is **RD-unstable of the first kind** if, for some choice of the parameters, its associated dispersion relation yields a finite amount of unstable wavenumbers (see Fig.6A-B). In this case, the resulting patterns, if heterogeneous, are periodic since series expansion (13) reduces to a sum of finitely many unstable eigenmodes, and eigenmodes are trigonometric functions for the vast majority of simple 1, 2 and 3-dimensional domains considered for pattern transformations in development (Baker et al., 2008). Notice that, since none of the initial patterns we consider are periodic, the emergence of these periodic resulting patterns constitutes a non-trivial pattern transformation (i.e. new concentration peaks and valleys will arise in some places).

We say that a gene network is **RD-unstable of the second kind** if, for some choice of the parameters, its associated dispersion relation yields an infinite amount of unstable wavenumbers (see Fig.6). Previous research (Klika *et al*., 2012) has shown that the dispersion relation never goes to positive infinity (see Fig.6E) and that gene networks that are RD-unstable of the second kind have a dispersion relation that saturates to a positive value and, thus, have an infinite queue of large unstable wavenumbers (see Fig.6C-D). Since there is an infinite number of unstable wavenumbers, the resulting patterns, if heterogeneous, can have an infinite sum of eigenmodes in (9). There exist both periodic and non periodic functions that admit such an infinite series representations (Stein & Shakarchi, 2003). Nevertheless, as we later explain, these gene networks lead to three broad types of heterogeneous resulting patterns: periodic (but not trigonometric) patterns, noisy patterns, and radially symmetric multi-peaked patterns. Which of these can arise depends on the initial pattern and the topological class of the gene network.

Positive regulatory loops determine the kind of RD-instability

For a gene network to be RD-unstable it needs to have one or more positive regulatory loops. If such loops are all extracellular, then the gene network can only be RD-unstable of the first kind or RD-stable ; and if such loops are all intracellular, then the gene network can only be RD-unstable of the second kind or RD-stable. In here, we provide a proof of these two statements for the particular case of gene networks with only one positive loop and linear degradation, while in S2 of the SI we give a general prove for gene networks with any number of loops.

In the square matrix $\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)$ in (13), each off-diagonal entry corresponds to the interaction between two different gene products, while each diagonal entry contains the variable λ, the degradation rate of a given gene product, its diffusion coefficient and, perhaps, some self-regulation term (i.e., $J_{ii}\neq 0$). By the Leibniz formula for determinants (Strang, 2016), each term in $det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right)$ (i.e., the characteristic polynomial of the reaction-diffusion matrix) is the product of N_g entries of different rows and columns (i.e. different permutations). This means that each term of the characteristic polynomial of $\boldsymbol{R}(\boldsymbol{k}^2)$ contains only one entry per gene product (i.e., row or column) and hence, only the terms that correspond to regulatory loops are non-zero (since all other terms contain at least one zero element in the product), except for the product of all diagonal entries. The characteristic polynomial reduces, thus, to a sum of loop terms. This implies that, if a gene network contains no loops, the characteristic polynomial of $\boldsymbol{R}(\boldsymbol{k}^2)$ has just one term: the product of the elements of its diagonal. Namely, the dispersion relation of the network reads,

$$\det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) = \prod_{i=1}^{N_g}\left(\lambda + \mu_i + \boldsymbol{k}^2 d_i\right) = 0, \tag{14}$$

and, thus, its eigenvalues are given by,

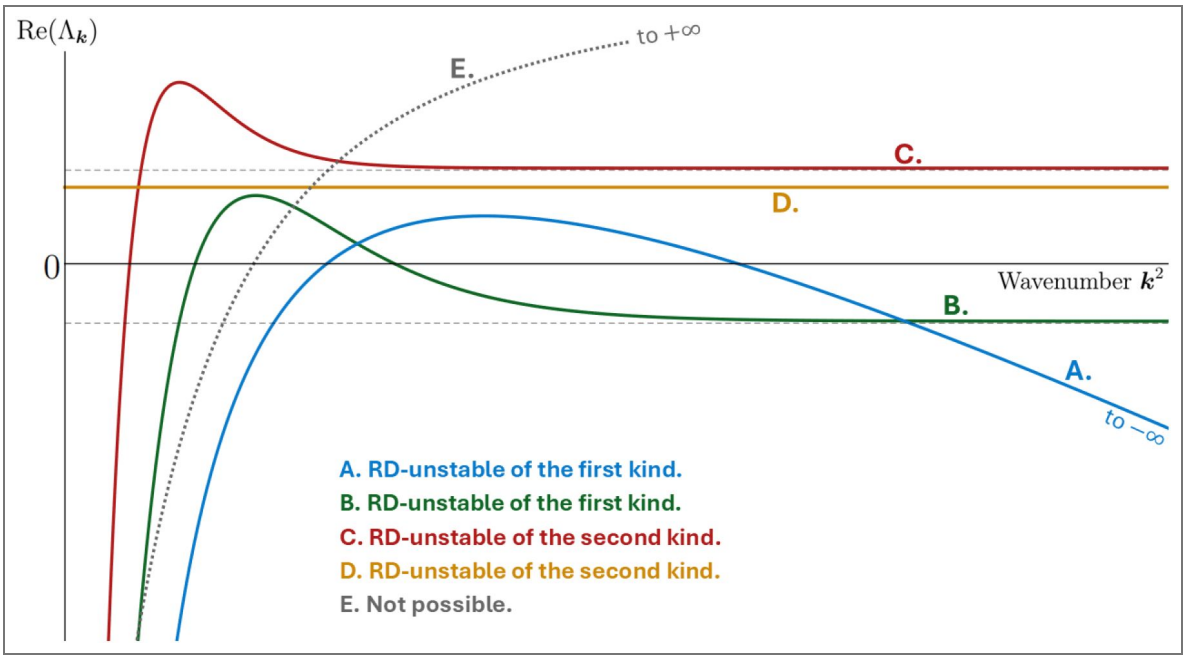


Figure 6. Instances of principal branches of dispersion relations.

(A-B) Dispersion relations of RD-unstable gene networks of the first kind (i.e. there is only a number of wavenumbers, x-axis, for the which there are eigenvalues with a positive real part). The real part of the principal branch of a dispersion relation can diverge to $-\infty$ for large wavenumbers **(A)**; it can converge to a negative finite value **(B). (C-D)** Dispersion relation of RD-unstable gene networks of the second kind (i.e. there is an infinite number of wavenumbers with eigenvalues with a positive real part). The real part of the principal branch of a dispersion relation cannot diverge to $+\infty$ (Klika *et al*., 2012).

$$\lambda_i(k) = -\mu_i - k^2 d_i, \tag{15}$$

for all i=1, ..., N_g. In this case, all eigenvalues are negative and, thus, all wavenumbers are stable: no pattern transformation is therefore possible. This proves that, without regulatory loops in the network, pattern transformations are not possible.

Let us now consider the case of a gene network with only one loop, which we assume to contain N_l>1 gene products. Without loss of generality, gene products can be relabeled so that the submatrix of $\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)$ of the loop contains all non-zero elements under the diagonal and in the top-right entry. Namely,

$$\begin{pmatrix} \lambda+\mu_1 & 0 & 0 & \dots & 0 & -J_{1N_l} \\ +\boldsymbol{k}^2 d_1 & & & & & \\ -J_{21} & \lambda+\mu_2 & 0 & \dots & 0 & 0 \\ & +\boldsymbol{k}^2 d_2 & & & & \\ 0 & -J_{31} & \lambda+\mu_3+\boldsymbol{k}^2 d_3 \dots & & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & \lambda+\mu_{N_l-1} & 0 \\ & & & & +\boldsymbol{k}^2 d_{N_l-1} & \\ 0 & 0 & 0 & \dots & -J_{(N_l-1)N_l} & \lambda+\mu_{N_l} \\ & & & & & +\boldsymbol{k}^2 d_{N_l} \end{pmatrix}. \tag{16}$$

Similarly, given that there exists no other loop in the network, we can relabel the remaining gene products so that the rest of the matrix is upper triangular (Bang-Jensen, 2008). Thus, the characteristic polynomial has two terms: the product of all diagonal entries, and the product of the non-diagonal entries in the loop times the remaining N_g–N_l diagonal entries. The corresponding dispersion relation reads,

$$\begin{aligned} &\det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) = \\ &\prod_{i=1}^{N_g}\left(\lambda+\mu_i+\boldsymbol{k}^2 d_i\right) + (-1)^{N_l+1}\left(-J_{1N_g}\right)\prod_{i=2}^{N_l-1}\left(-J_{i(i-1)}\right) \\ &\prod_{i=1}^{N_g-N_l}\left(\lambda+\mu_i+\boldsymbol{k}^2 d_i\right) = 0, \end{aligned} \tag{17}$$

where the minus sign before each J_{ij} comes from (13) and the factor $(-1)^{N_g+1}$ comes from the Leibniz formula for the case of a loop like (16). A (−1) factor can be extracted from each Jacobian entry to obtain a $(-1)^{N_l}$ factor that then simplifies (17) to,

$$\begin{aligned} \det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) &= \prod_{i=1}^{N_g}\left(\lambda+\mu_i+\boldsymbol{k}^2 d_i\right) \\ -J_{(1,N_g)}\prod_{i=2}^{N_1-1}J_{(i,i-1)}&\prod_{i=1}^{N_g-N_1}\left(\lambda+\mu_i+\boldsymbol{k}^2 d_i\right) = 0. \end{aligned} \tag{18}$$

Similarly, the factor $(\lambda+\mu_i+\boldsymbol{k}^2 d_i)$ is found N_g times in the left term and N_g–N_l times in the right term and so, (18) can be further simplified into,

$$\det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) = \prod_{i=1}^{N_1}\left(\lambda+\mu_i+\boldsymbol{k}^2 d_i\right) - J_{(1,N_g)}\prod_{i=2}^{N_1-1}J_{(i,i-1)} = 0. \tag{19}$$

All terms in the first product of (19) are positive and, thus, its expansion is an N_g-polynomial in λ with positive coefficients,

$$\det\left(\lambda \boldsymbol{I}_{N_g} - \boldsymbol{R}\left(\boldsymbol{k}^2\right)\right) = \lambda^{N_g} + \ldots + \prod_{i=1}^{N_l}\left(\mu_i + \boldsymbol{k}^2 d_i\right) - J_{(1,N_g)}\prod_{i=2}^{N_l} J_{(i,i-1)} = 0; \tag{20}$$

and then, the characteristic polynomial has a negative constant term if:

$$J_{(1,N_g)}\prod_{i=2}^{N_l} J_{(i,i-1)} > \prod_{i=1}^{N_l}\left(\mu_i + \boldsymbol{k}^2 d_i\right). \tag{21}$$

Notice condition (21) only holds if the regulatory loop is positive (i.e. all regulations are positive or there is an even number of negative ones). If condition (21) holds for some $\boldsymbol{k}$, it follows that the characteristic polynomial takes a negative value for λ=0. Moreover, since the term with the largest degree is positive, the characteristic polynomial becomes positive as $\lambda \to \infty$. This implies that the polynomial has to cross the λ-axis at least at one positive λ and such positive root is a positive eigenvalue of $\boldsymbol{R}(\boldsymbol{k}^2)$ for any $\boldsymbol{k}$ satisfying (21). In other words, we have proven that the dispersion relation of the gene network with a positive regulatory loop can yield unstable wavenumbers and, thus, the network can be RD-unstable depending on the parameters.

If the positive regulatory loop is extracellular, then at least one of the diffusion coefficients of its gene products is non-zero (i.e., $d_i \neq 0$ for some i in (21)). Then, given any specific choice of the parameters (i.e., specific values for J_{ij}) condition (21) will not hold for large wavenumbers $\boldsymbol{k}$ because the right-hand side simply grows unbounded as $\boldsymbol{k}^2 \to \infty$. This means that condition (21) can only hold, at most, for a finite amount of small wavenumbers $\boldsymbol{k}$. Thus, gene networks that have a single positive loop and in which this loop is extracellular, can only be RD-unstable of the first kind, or not RD-unstable at all. A general proof for the case of gene networks with several positive extracellular loops, and in fact for any gene network topology, is given in S2 of the SI.

If the positive loop is intracellular, then the diffusion coefficients of all its gene products are zero (i.e., d_i=0 for all i in (21)). Consequently, condition (21) reduces to,

$$J_{(1,N_g)}\prod_{i=2}^{N_l} J_{(i,i-1)} > \prod_{i=1}^{N_l}\mu_i. \tag{22}$$

In this case, the existence of positive eigenvalues does not depend on any specific wavenumber $\boldsymbol{k}$. This means that, whenever the parameters allow (22) to hold, all wavenumbers $\boldsymbol{k}$ are unstable (i.e., there are infinitely many unstable wavenumbers). In other words, gene network that have a single intracellular positive loop can only be RD-unstable of the second kind, or not RD-unstable. Again, a general proof for the case of gene networks with several positive intracellular loops is given in S2 of the SI.

As we have seen, at least one positive regulatory loop is a necessary for a gene network to lead to pattern transformation. As we will see, for non-trivial pattern transformations to occur, negative regulation is also required (otherwise, the resulting pattern can only be homogeneous). Given that positive loops are required, and that there are only three classes of signal subnetworks, it follows that the gene networks leading to pattern transformation can only be: gene networks with positive intracellular loops and intracellular negative regulation (i.e., H networks); gene networks with positive intracellular loops and negative extracellular loops (i.e., L^- gene networks with positive intracellular loops l^+); gene networks with extracellular positive loops and extracellular negative loops (i.e., emergent L^+L^- gene networks); or a combination of any of the above. As we will see, each of these classes of gene networks leads to qualitatively different types of resulting patterns and the dispersion relation, together with some topological considerations, can be used to shed some light into the types of resulting patterns possible.

Hierarchical gene networks

By definition, hierarchical networks do not have extracellular loops and, due to requirement I2, hierarchical gene networks leading to non-trivial pattern transformations include at least one positive intracellular loop. As we saw in the previous section, this means that these networks are RD-unstable of the second kind.

Pattern transformations from spike initial patterns in H networks with a single extracellular signal

Let us begin by considering H gene networks consisting of a single H subnetwork with a single extracellular signal. We call these H^0 networks. From a spike initial pattern, it is only the cells in the spike that, by definition, express the extracellular signal. This implies that it is only these cells that are secreting the extracellular signal. Thus, the concentration of the extracellular signal in all other cells (i.e., cells outside the spike) is fully governed by signal diffusion from this source and its degradation. Indeed, if we consider the set of cells either on the right-hand side of the spike (computations are equivalent for the left-hand side), we can solve equation (1) assuming linear degradation in the signal to show that its concentration forms an exponential gradient around the spike,

$$g_A^*(x) = Ce^{-\sqrt{\frac{\mu_A}{d_A}}(x-L)}, \text{ for some constant } C > 0, \tag{23}$$

where A is the extracellular signal in the H^0 network, g_A^* is its concentration in the resulting pattern, x is the distance to the border of the spike and L is half the width of the spike. In 2D, the signal concentration gradient decays as $\sim e^{-\|\boldsymbol{x}\|_2}/\sqrt{\|\boldsymbol{x}\|_2}$, where $||\boldsymbol{x}||_2$ denotes euclidean norm; and in 3D, as $\sim e^{-\|x\|_2}/\|x\|_2$ (Sommerfeld, 1949; Stakgold & Holst, 2011). In all cases signal concentration has a radially decaying spatial distribution centered on the spike. In S3 of the SI we calculate C.

In the spatial distribution arising from (23), each cell at each side of the spike is at a unique distance from the spike and, thus, experiences a unique concentration of A. In that sense, there is a unique correspondence between the concentration of A and the distance to the spike. This correspondence has been used in the literature (Wolpert, 1968) to propose that, with a single extracellular signal, any pattern transformation is possible (see Fig.S1 in the Supplementary Information). The only thing that would be required is that cells interpret the signal concentration in a different way for each different resulting pattern attained (Capek and Müller, 2019; Sharpe, 2019). However, what would that interpretation be, or which would be its underlying gene network, was not specified by this author (Horder, 2001). Allegedly, any interpretation should rely on specific gene networks. A common idea for such interpretation is 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 from the spike and, thus, a non-trivial pattern transformation would occur (Capek and Müller, 2019; Sharpe, 2019).

There are, however, many other ways in which H^0 networks can lead to non-trivial pattern transformations. In the following, we explain some fundamental requirements that H^0 networks need to fulfill in order to lead to non-trivial pattern transformations and which are their possible non-trivial pattern transformations. This new requirements should be added on top of requirements R1-R5 to enable non-trivial pattern transformations in H^0 networks.

Requirement RH1

For non-trivial pattern transformations in H^0 networks, at least one gene product that is positively downstream of the extracellular signal A (let us say gene product k) must inhibit at least one gene product that is also positively downstream of A (let us say gene product j).

Requirement (RH1) has been previously discussed by other authors (Munteanu *et al*., 2014), especially for networks with three gene products, but we include it and expanded it here for completeness (see Fig.7). This requirement can be split into two parts: 1) there needs to be at

least two gene products that are positively downstream of A; and, 2) one of them has to inhibit the other. Part 1 is simply requirement I1 applied to H^0 networks: any gene product undergoing a non-trivial pattern transformation must be positively downstream of an extracellular signal and in H^0 networks, this is A because it is the only signal there is.

To understand part 2, let us first consider H^0 networks in which part 1 holds but part 2 does not i.e., j and k are both positively downstream of A, but j is not inhibited by k. In that case, according to requirement R5 on $\boldsymbol{f}$, both k and j will increase their concentration when that of A increases, and decrease their concentration when that of A decreases. Since the concentration of A decreases away from the spike (23), it follows that the concentrations of k and j also decrease with the distance to the spike and thus, k and j have their concentration peak in the same place as A and, thus, no non-trivial pattern transformation occurs. In fact, the same will occur for any gene products that, in a H^0 network, are positively, and only positively, downstream of A.

Let us now consider the case in which part 1 and part 2 of RH1 hold. In that case, there is at least one gene product, k, that is downstream from A and inhibits another gene product, j, that is also downstream from A. In this case, the concentration of j can become very small where that of k is large (i.e., in the spike) and thus, form a concentration valley centered in the spike. This valley will in turn be flanked by two concentration peaks of j at each side (see Fig. 8). In the 2D and 3D cases there would be a central basin and a ridge surrounding it (see Fig.8 and Fig.9). However, the emergence of these concentration peaks and valleys is only possible if function $\boldsymbol{f}$ fulfills at least one of the two following additional requirements (see Fig.7):

Requirement RH2a

function $\boldsymbol{f}$ is such that the proportion between the activations of k and j by A changes with the concentration of A (i.e., $f_k(g_A, \ldots)/ f_j(g_A, \ldots)$ is not a constant function of g_A). In other words, the functional form of the activation of k by A (i.e., $f_k(g_A, \ldots)$) has a different non-linear part than the functional form of the activation of j by A (i.e., $f_j(g_A, \ldots)$).

Requirement RH2b

The inhibition of j by k is non-linear in the sense that it is disproportionately larger for high concentrations of k than for low concentrations of k.

Both requirements imply an inhibition of j that is disproportionately different for different values of g_A and, thus, for different distances to the spike. In RH2a, this disproportionality arises from the different non-linear parts of the activations of j and k by A, while in RH2b, it arises from an intrinsic non-linearity in the inhibition of j by k (see Fig.7).

To better understand these requirements lets consider what happens if neither RH2a nor RH2b hold and all relevant interactions are linear. In this case, gene products k and j have concentrations that are, in each cell, proportional to those of A (and, thus, proportional to each other). In this case, if k inhibits j linearly, then g_j decreases proportionally to g_k in each cell. Consequently, g_j remains proportional to A over space but it is simply expressed at a lower overall level (i.e., no non-trivial pattern transformation can occur).

If the inhibition of j by k is disproportionately higher when g_k is large i.e. non-linear inhibition, then g_j would be disproportionately smaller where g_k is large (i.e. around the spike) than where g_k is small (i.e. away from the spike). This can lead to the formation of a valley of g_j in and around the spike and, consequently, to the formation of two peaks of concentration around it (in two or higher dimensions that would be a basin and a ridge surrounding it, see Fig.9).

To illustrate these arguments lets us consider an example gene network and $\boldsymbol{f}$ in which signal A directly activates gene products j and k, and k directly inhibits j in such a way that j and k are regulated by no other gene product in the network. In H^0 networks the concentration of A is given by equation (23) and the dynamics of j and k are given by (24).

$$\begin{cases} \partial_t g_k(t,x) = f_k\left(g_A(t,x)\right) - \mu_k g_k(t,x) \\ \partial_t g_j(t,x) = f_j\left(g_A(t,x), g_k(t,x)\right) - \mu_j g_j(t,x) \end{cases} \tag{24}$$

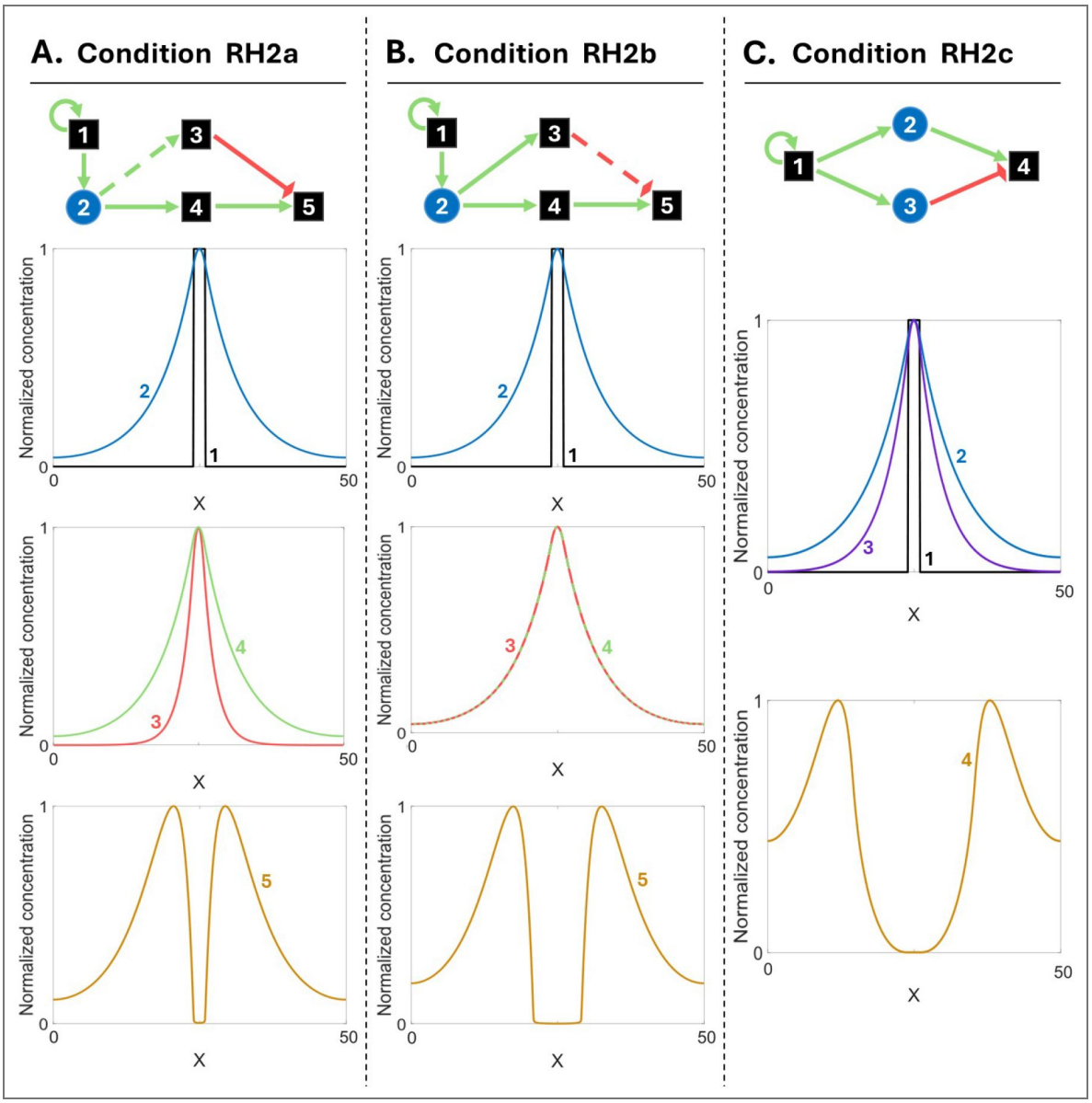


Figure 7. Diagram showing the topological requirements for pattern transformation in hierarchical gene networks

using example gene networks. (A) Requirement (RH2a): the positive regulation of 3 by *A* (discontinuous green arrow) has a different non-linearity than that of 4 by *A*. (B) Requirement (RH2b): the negative regulation of 5 by 3 (discontinuous red arrow) has a different non-linearity than that of 5 by 4. (C) Requirement (RH2c): two extracellular signals downstream of 1 and upstream of 4. The signals have different concentration profiles over space because either they have different diffusion coefficient or different intrinsic degradation rates. Each of these requirements lead to the formation of two new concentration peaks in the most downstream gene product. Network colors and shapes as in Figure 2. Simulations were run using a Forward-Euler algorithm on the Maini-Miura model (see S6 above for parameter values). Notice that in **(A)** gene product 3 is an example of whatcall gene product *j* in the main text, while in **(B)** gene product *j* corresponds to gene product 5.

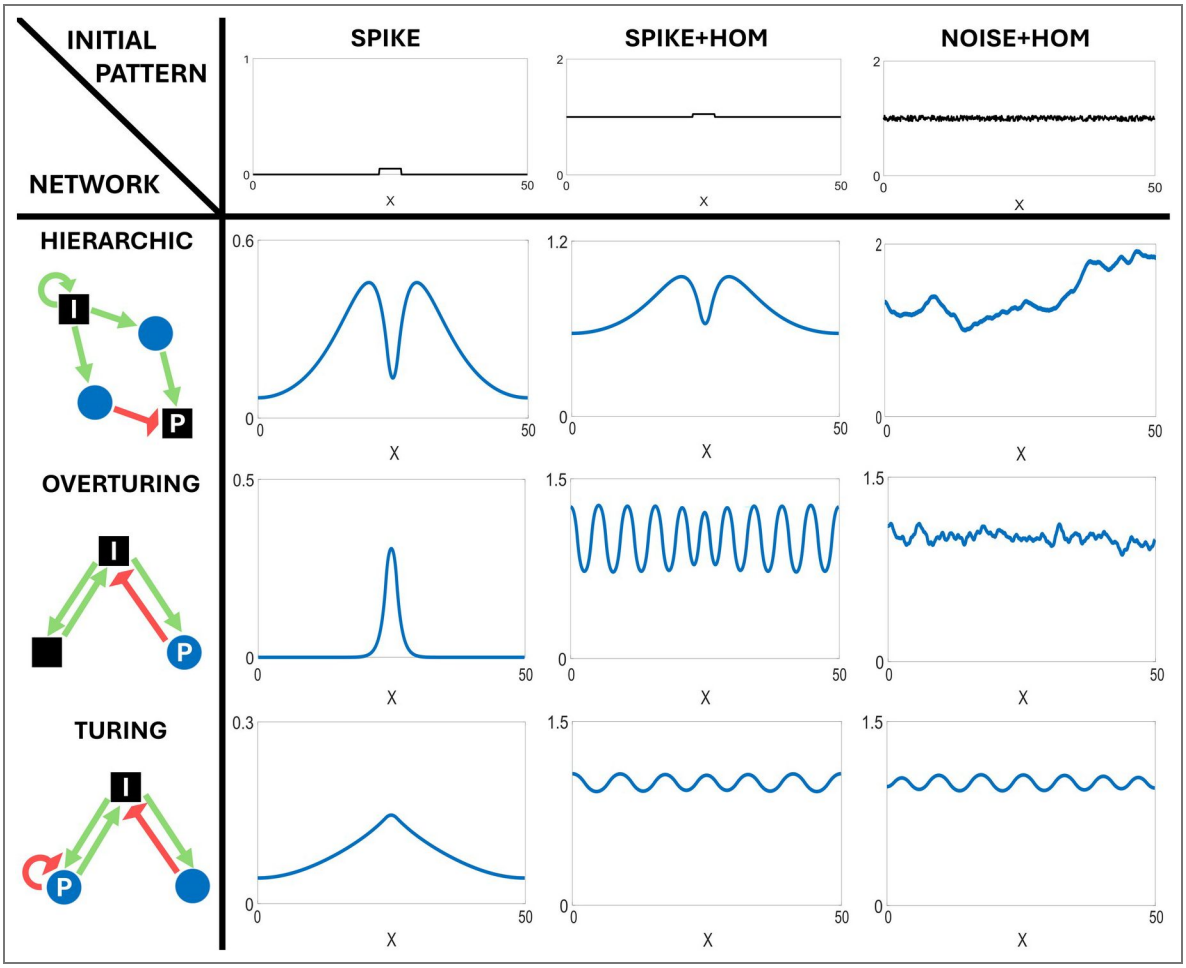


Figure 8. Classes of gene networks capable of pattern formation and their resulting patterns in 1D.

The first column depicts simple examples of each class 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 topologies. The network topologies in the left column are only simple ones, for a more detailed description of the possible ones check the main text. For the over-Turing topology we chose to represent a intracellular loop with two gene products (but we could have chosen one). Note that some of the pattern transformations are trivial (over-Turing and Turing on spike initial pattern). H network can lead to non-trivial pattern transformations from homogeneous-with-noise initial patterns. Network colors and shapes as in Figure 2, except that *P* stands for the gene product plotted as resulting pattern while *I* stands for a gene product present in the initial pattern. Simulations were run using a Forward-Euler algorithm on the Maini-Miura model for $\boldsymbol{f}$ (see S6 in SI for parameter values).

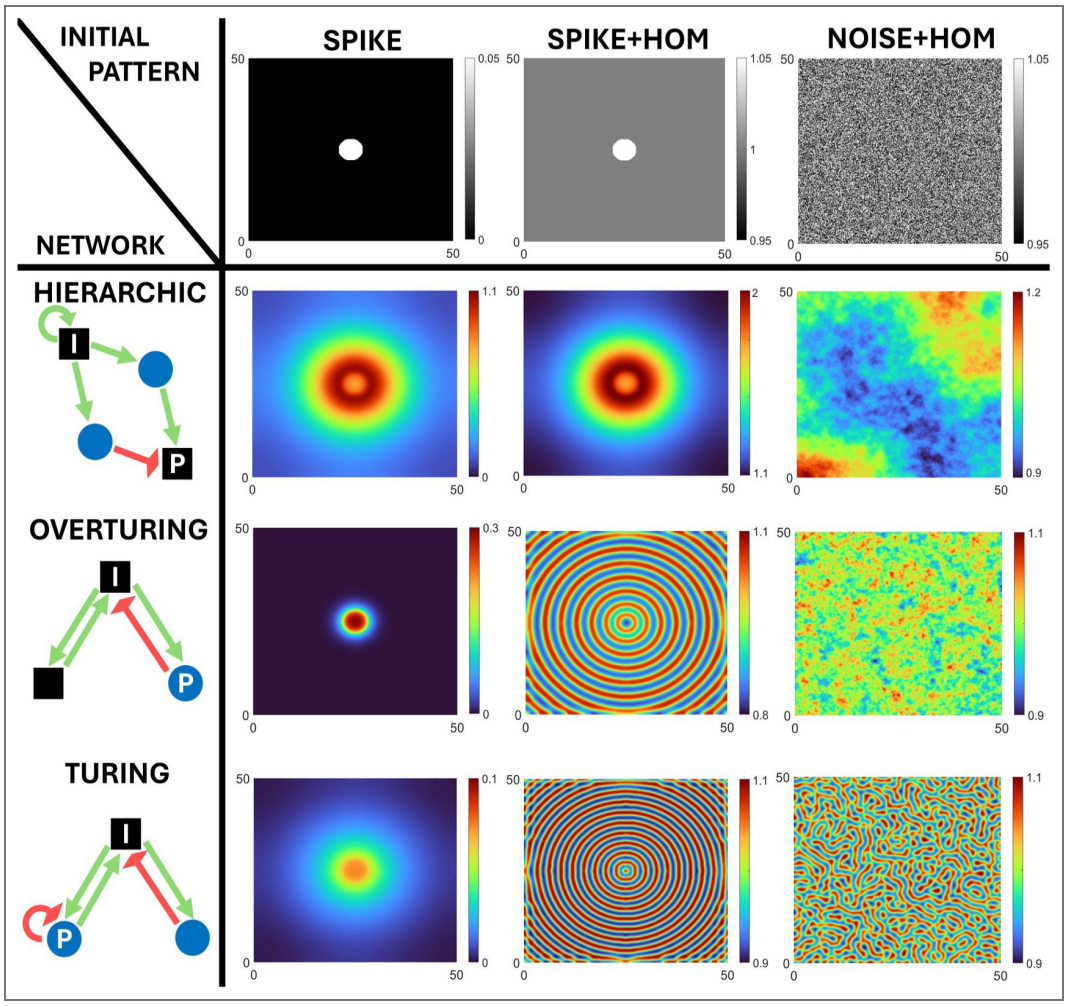


Figure 9. Classes of gene networks capable of pattern formation and their resulting patterns in 2D.

The first column depicts simple examples of each class 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 topologies. In the case of spike and spike-homogeneous initial patterns, the resulting patterns correspond to the revolution of the patterns in Figure 8 around the center of the spike given the radial symmetry of equation (1) and the spike. In homogeneous-with-noise initial patterns, the white noise breaks such symmetry. In the case of Turing networks acting on homogeneous-with-noise initial pattern other types of periodical resulting patterns (e.g., dots or stripes) have been reported in the literature (Murray, 2002). Network colors and shapes as in Figure 8, Simulations were run using a Forward-Euler algorithm on the Maini-Miura model for $\boldsymbol{f}$ (see S6 in SI for parameter values).

Where we assume, for simplicity, that both j and k suffer linear degradation (i.e., m_j=m_k=1). Now, assume that regulations $f_k(g_A)$ and $f_j(g_A, g_k)$ are both linear. Then, system (24) reads,

$$\begin{cases}\partial_t g_k(t,x) = B_{kA} g_A(t,x) - \mu_k g_k(t,x), \\ \partial_t g_j(t,x) = B_{jA} g_A(t,x) - B_{jk} g_k(t,x) - \mu_j g_j(t,x),\end{cases} \tag{25}$$

where B_{kA}, B_{jA}, B_{jk} >0 are real positive constants. Given that we are only interested in stationary resulting patterns, we can take time derivatives in (25) to zero and then we find that the concentrations of k and j in the resulting pattern are given by,

$$g_k^*(x) = \frac{B_{kA}}{\mu_k} C e^{-\sqrt{\frac{\mu_A}{d_A}}x}, \text{ and } g_j^*(x) = \left(\frac{B_{jA}}{\mu_j} - \frac{B_{kA}B_{jk}}{\mu_k \mu_j}\right) C e^{-\sqrt{\frac{\mu_A}{d_A}}x}, \tag{26}$$

where we have used the gradient shape of $g_A^*(x)$ from (23). Then, as we can see in (26), both concentrations have negative exponential distributions in space with the same exponential decay but different heights (i.e., different leading coefficients). Moreover, equation (26) also shows that there is a single concentration peak for A, j and k, and that these peaks are all centered at the same position (i.e. the center of the spike) and, thus, there is no non-trivial pattern transformation. This proves that no non-trivial pattern transformations are possible if all regulations are linear (requirement R2).

Now, let us consider the case in which all regulations remain linear except for the inhibition of j by k (as requested by RH2b). Then, system (24) becomes,

$$\begin{cases}\partial_t g_k(t,x) = B_{kA} g_A(t,x) - \mu_k g_k(t,x), \\ \partial_t g_j(t,x) = B_{jA} g_A(t,x) - f_{jk}\left(g_k(t,x)\right) - \mu_j g_j(t,x),\end{cases} \tag{27}$$

where f_{jk} is a non-linear inhibition of j by k. Hence, taking time derivatives in (27) equal to zero, the resulting pattern concentrations of j by k are,

$$\begin{aligned} g_k^*(x) &= \frac{B_{kA}}{\mu_k} C e^{-\sqrt{\frac{\mu_A}{d_A}}x}, \text{ and } g_j^*(x) \\ &= \frac{B_{jA}}{\mu_j \mu_k} C e^{-\sqrt{\frac{\mu_A}{d_A}}x} - \frac{1}{\mu_j} f_{jk}\left(\frac{B_{kA}}{\mu_k} C e^{-\sqrt{\frac{\mu_A}{d_A}}x}\right), \end{aligned} \tag{28}$$

where we have used the gradient shape of $g_A^*(x)$ from (23). For gene product k, we have the same result as before: a single concentration peak centered in the spike. Things are, however, different for the resulting pattern of gene product j. From requirement R5, we know that f_{jk} is monotonically increasing with g_A and hence, it decreases with x. The two terms in $g_j^*(x)$ have a maximum at x=0 and then, since the second term is negative, the overall value of $g_j^*(x)$ in (28) can be lower at x=0 than elsewhere. If this happens, a concentration valley of j can form at x=0, flanked by two concentration peaks of j at each side of it. This implies the formation of new concentration peaks and valleys, and thus, that non-trivial pattern transformations are possible (see Fig.7) when requirement (RH2b) holds.

Next, let us consider the case in which RH2a holds and RH2b does not. In this case, the activation of k by A at high concentrations of A is disproportionately larger than that of j by A. As a result, g_k would be disproportionately larger than g_j in, and close to, the spike and not away from it (i.e. g_k and g_j would decay with a different slope from the spike). Then the inhibition of j by k, even when linear, can lead to the formation of a valley of g_j in and around the spike and, consequently, to the formation of two concentration peaks away from the spike (in two and higher dimensions that would be a concentration ridge and basin, see Fig.9).

Let us illustrate these arguments with a specific example in which regulations $f_k(g_A)$ and $f_j(g_A, g_k)$ are non-linear, but the activations of *k* and *j* by *A* have a different non-linear part in their functional forms (as required by RH2a) In this case, system (24) reads,

$$\begin{cases} \partial_t g_k(t,x) = f_k\left(g_A(t,x)\right) - \mu_k g_k(t,x), \\ \partial_t g_j(t,x) = f_{jA}\left(g_A(t,x)\right) - B_{jk} g_k(t,x) - \mu_j g_j(t,x), \end{cases} \tag{29}$$

where $f_{jA}(g_A)$ is a non-linear activation of *j* by *A* that is different from that of $f_k(g_A)$ (e.g., one is a quadratic monomial and the other, a cubic monomial). We maintain linear inhibition of *j* by *k* because we are considering the case in which RH2a holds but RH2b does not. As in previous cases, if we equal time derivatives in (29) to zero, we get that the stationary resulting patterns of *j* and *k* are given by.

$$\begin{aligned} g_k^*(x) = \frac{1}{\mu_k} f_k\left(Ce^{-\sqrt{\frac{\mu_A}{d_A}}x}\right), \text{ and } g_j^*(x) \\ = \frac{1}{\mu_j}\hat{f}_{jA}\left(Ce^{-\sqrt{\frac{\mu_A}{d_A}}x}\right) - \frac{B_{jk}}{\mu_j} f_k\left(\frac{B_{kA}}{\mu_k} Ce^{-\sqrt{\frac{\mu_A}{d_A}}x}\right), \end{aligned} \tag{30}$$

where we have used (23). Requirement R5 ensures that $f_k(g_A)$ and $f_{jA}(g_A)$ are both monotonically increasing functions of g_A and consequently, monotonically decreasing functions of *x*. For gene product *k*, this means that its resulting pattern will have the same monotony as $g_A^*(x)$ (including its unique maximum at *x*=0), but with a different slope. Similarly, the two terms in $g_j^*(x)$ share that same monotony with a maximum at *x*=0 but different slopes (e.g., if $f_k(g_A) = B_{kA} g_A^2$ and $f_{jA}(g_A) = B_{jA} g_A^3$, then $g_j^*(x) \sim e^{-(\omega_A/2)x} - e^{-(\omega_A/3)x}$, with $\omega_A = \sqrt{\mu_A/d_A}$. Consequently, since the second term is negative, the overall value of $g_j^*(x)$ in (30) can be lower at *x*=0 than it is in nearby regions and, if this happens, a concentration valley of *j* can form at *x*=0, flanked by two concentration peaks of *j* at each side of it. Like before, when and where these concentration peaks and valleys form depends on the specific choice of the parameters (again, the parameters choice must be such that $g_j^*(x) \geq 0$ for all *x*). However, if they form, then gene product *j* would undergo a non-trivial pattern transformation as two new concentration peaks emerge at positions where there was no previous concentration peak (see Fig.7). This proves that requirement (RH2b), together with the proper choice of the parameters, enables non-trivial pattern transformations in H^0 networks.

It is worth mentioning that, although we used a very simple H^0 network for the illustration of requirements RH2a and RH2b, both requirements apply even if the regulations involved are not direct (e.g., if *k* activates some intermediate gene product *i* and *j* activates *j*). In this case, the different non-linear part in the functional form of regulations can be in any intermediate regulation (e.g., in the activation that *k* exerts on *i*, or the one that *i* exerts on *j*).

Pattern transformations from spike initial patterns in general H networks

In general, H networks may have different extracellular signals and these may affect patterning in downstream gene products. These networks can also lead to non-trivial pattern transformations and they do it with a set of less restrictive requirements than H^0 networks (see Fig.7C). In this case, inhibition does not need to occur between gene products downstream of the same extracellular signal *A*, but can occur between gene products downstream from different extracellular signals secreted from the spike, e.g. signals A_1 and A_2. Then, requirements RH1, RH2a and RH2b are no longer necessary as long as these different extracellular signals have different diffusion coefficients (i.e., $d_{A_1} \neq d_{A_2}$), or different degradation coefficients (i.e., $\mu_{A_1} \neq \mu_{A_2}$). Hence, former additional requirements shall be substituted by the following one:

Requirement RH2c

For non-trivial pattern transformations H networks with several extracellular signals, there must exists some gene product *j* that is positively downstream of one extracellular signal A_1 and negatively downstream of another extracellular signal A_2, both secreted from the spike and with different diffusion coefficients (or different degradation rates).

If this occurs, then according to (23), these two signals have concentration gradients with different slopes along x. In other words, some of the extracellular signals 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 Fig.7). To illustrate this possibility, let us consider a simple H network where extracellular signal A_1 directly activates an intracellular gene product *j*, while signal A_2 directly inhibits it. Thus, if we assume that regulation $f_j\left(g_{A_1}, g_{A_2}\right)$ is linear, the concentration dynamics of *j* is given by,

$$\partial_t g_j(t,x) = B_{jA_1} g_{A_1}(t,x) - B_{jA_2} g_{A_2}(t,x) - \mu_j g_j^{m_j}(t,x), \tag{31}$$

where B_{jA1},B_{jA2}>0 are positive real constants. Then, taking the time derivative in (31) equal to zero, we have that the emerging patter of *j* is given by,

$$g_j^*(x) = \frac{1}{\mu_j}\left(B_{jA_1} C_{A_1} e^{-\sqrt{\frac{\mu_{A_1}}{d_{A_1}}}x} - B_{jA_1} C_{A_2} e^{-\sqrt{\frac{\mu_{A_2}}{d_{A_2}}}x}\right)\frac{1}{m_j}, \tag{32}$$

Equation (32) is essentially the difference of two negative exponential functions. Then, if the slope of the first exponential is smaller than that of the second exponential, this subtraction can lead to the formation of concentration valley in $g_j^*(x)$ at *x*=0 flanked by two concentration peaks at each side of it (thus leading to non-trivial pattern transformation).

Pattern transformations arising from the combination of multiple H subnetworks

Gene products that undergo a non-trivial pattern transformation through an H network can lead to further non-trivial pattern transformations in downstream gene products without the need for activating additional extracellular signals (Scalise & Schulman, 2014). This can occur in gene networks in which gene products with a heterogeneous resulting pattern activate a gene product that is, in turn, inhibited by gene products with another different pattern (see Fig. 10). The resulting pattern in the downstream gene product can then have concentration valleys where its inhibitory gene products have concentration peaks, and concentration peaks where its activating gene products have concentration valleys (we call this peak subtraction).

Similarly, a gene product that is positively downstream of gene products with different patterns can also develop a new pattern in which concentrations peaks would form wherever any of the upstream gene products have concentration peaks, as long as the concentration peaks of upstream activators are sufficiently distant from one another, (see Fig.10). We call this peak addition.

In both cases, the downstream gene product may acquire new concentration peaks and valleys and, thus, peak addition and subtraction can lead to non-trivial pattern transformations. The total number of peaks in the resulting pattern is less than or equal to the sum of the number of peaks in the resulting pattern of upstream gene products (as explained in section S4 and S5 of SI).

The ensemble of possible pattern transformations from spike initial patterns in H networks

As we have seen in the previous subsection, H subnetworks can be combined in simple ways to lead to resulting patterns with many concentration peaks and valleys. In this subsection we argue that, with some broad restrictions, for any radially symmetric resulting pattern there is a H network producing it from a spike initial pattern.

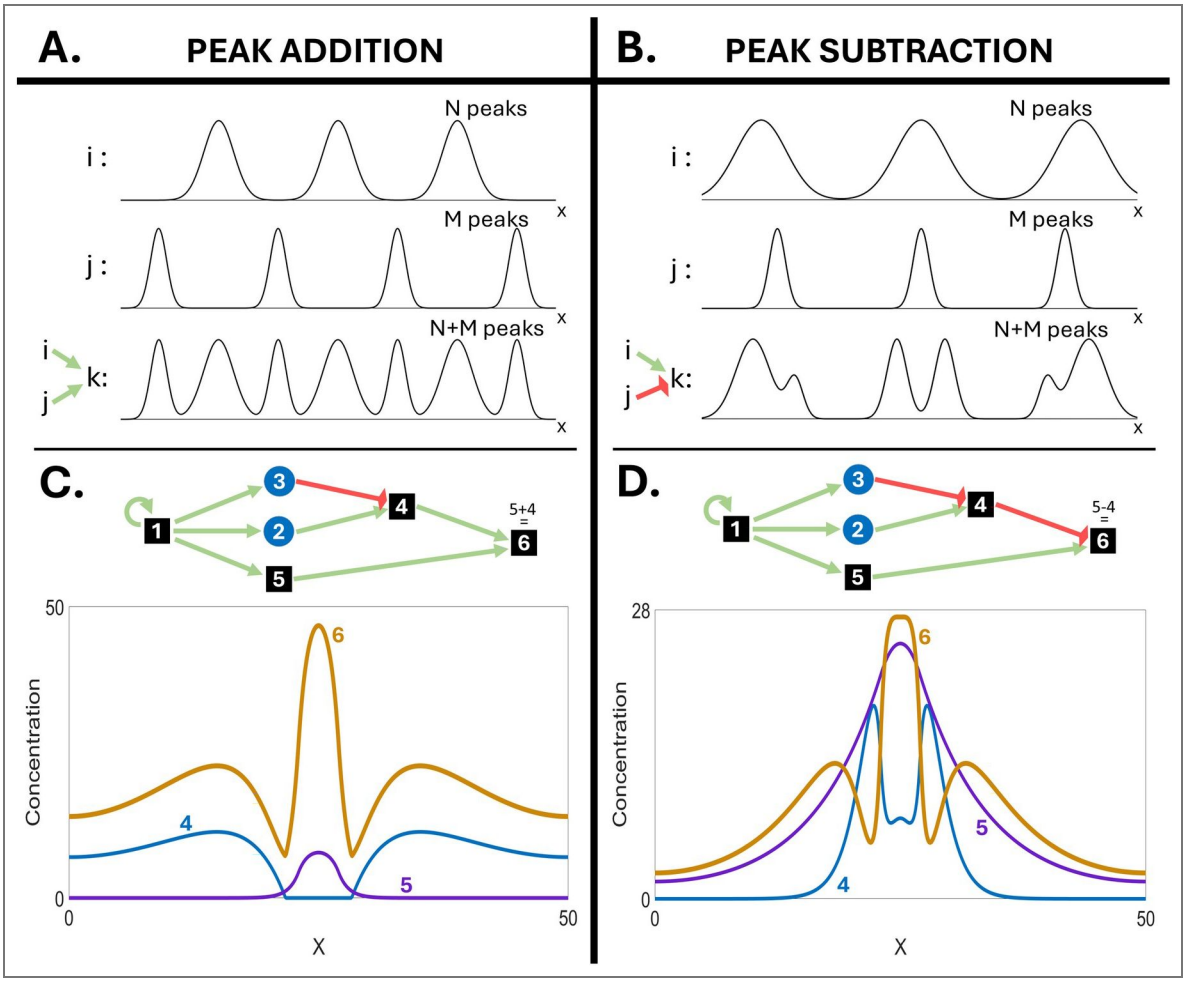


Figure 10. Peak addition and subtraction in the resulting pattern of downstream gene products from hierarchic gene networks.

The most upper row shows an idealized pattern of a gene product *i* with several concentration peaks. The middle row that of another gene product, *j*, with several concentration peaks. In **(A)** both gene products positively regulate the same gene product, *k*. As a result gene product *k* has concentration peaks either *i* or *j* have them. In **(B)**, instead, *j* inhibits *k* while *i* activates it. **(C)** shows an example network in which the pattern of two gene products, 4 and 5, are effectively added into that of gene product 6. **(D)** shows and example gene network in which the patterns of gene products 4 is effectively subtracted from that of gene product 5 to give rise to the pattern of gene product 6. Simulations were run using a Forward-Euler algorithm on the Maini-Miura model for $\boldsymbol{f}$ (see S6 in SI for parameter values).

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 (or basins and ridges in higher dimensions). Now, let us consider a simple gene network that we call the diamond network (see Fig.11A). This gene network has an intracellular gene product in the spike (say gene product 1) that self-activates and activates two extracellular signals (say signals 2 and 3) that, in turn, regulate a downstream intracellular gene product (say gene product 4). Gene product 4 is activated by signal 2 and inhibited by signal 3. Finally, the gene network includes an intracellular gene product 5 that is activated by gene product 4.

As we know from previous subsections, if extracellular signals 2 and 3 have different diffusion coefficients, or degradation rates, the resulting pattern of 4 can exhibit a pair of concentration peaks at a distance from the spike. With some very mild restrictions (specified in S4 of SI), this distance can be made larger or smaller by changes in these parameters and the rest of parameters (see S4 of the SI for a more formal discussion) (see Fig.11). The resulting pattern of gene product 5 will exhibit concentration peaks in the same place as gene product 4. However, by choosing an adequate $f_5(g_4)$ (while keeping R1 to R5) the concentration peaks of gene product 5 can have different heights and steepness (i.e., different decrease rates from its peak). In the most extreme case (e.g., using a sigmoidal function for $f_5(g_4)$), the concentration of gene product 5 can be made to be only appreciable in the cell where it attains its peak and its most immediate neighboring cells (i.e. very narrow concentration peak).

This simple diamond gene network can be combined any number of times to produce any number of peaks in any combination of positions and heights: simply, gene product 1 would activate different diamond gene networks and each such networks would have different parameters that lead to concentration peaks in different locations and with different heights and steepness. Then, the peak addition described above (see Fig.10) can put together all such peaks in the resulting pattern of a gene product downstream of all such diamond networks. Hence, the resulting pattern can produce any array of concentration peaks and valleys (i.e. target resulting pattern) as long as it is symmetric around the spike, it is continuous over space, and peaks and valleys are sufficiently distant from each other (peaks that are very close together may merge; see S4 of SI). Moreover, it is worth noting that if the resulting pattern consists of very narrow concentration peaks, function $\boldsymbol{f}$ and its parameters may need to be tuned very precisely and thus, patterns made of extremely narrow peaks may not be biologically feasible in practice.

The hierarchical gene networks studied in the previous sections are just simple examples. Since these simple H gene networks can lead to nearly all resulting patterns, one can expect that there are other, more complex, H networks that can also do it.

Finally, it is worth noting that hierarchical gene networks acting on a spike initial pattern tend to have similar variational properties (as studied in Salazar-Ciudad et al., 2000, 2001). Thus, in many H networks, the properties of the different peaks and valleys in a pattern (i.e. their height, position and steepness) are able to vary in respect to those of many other peaks in a pattern (while keeping radial symmetry). In the combination of diamond networks just described, for example, each pair of peaks arises from a specific diamond subnetwork with specific extracellular signals and its own parameters. This implies that it is possible, as we have seen, to regulate the height, steepness, and position of each pair of peaks in the resulting patterns (or, of each ridge in higher dimensions) independently from each other through changes in the parameters of each subnetwork (see Fig. S2 for other simple examples). However, not in all H gene networks all peaks can vary independently from each other. Peak addition and subtraction, for example, can lead to some peaks varying together with parameter changes (Salazar-Ciudad *et al.*, 2000).

Pattern transformations from combined spike-homogeneous initial patterns in H networks

The pattern transformations possible from the combined spike-homogeneous initial patterns are similar to those possible from the spike initial conditions and have the same properties (see Fig.8-9). It suffices to add steady-state concentration $g_A^* \neq 0$ to the signal gradient in (23) and all subsequent arguments in the previous subsections follow similarly.

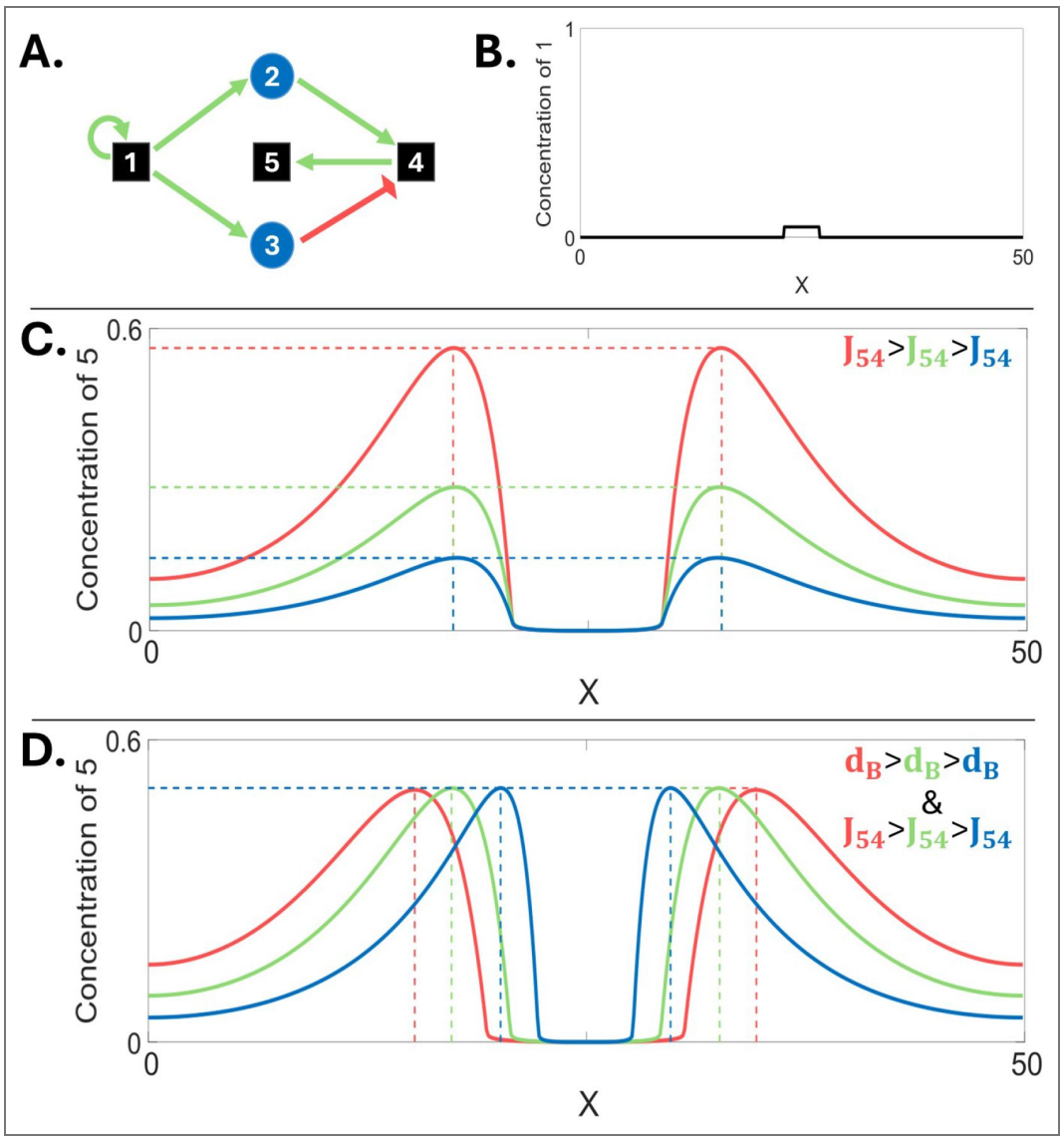


Figure 11. Variational properties of the diamond H network.

(A) Diamond H network (see S4 in SI). Network colors and shapes as in Figures 2 and 4. **(B)** Initial pattern in gene product 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. Peak height can be varied by changing a single parameter, that is the activation of gene product 5 by gene product 4 (i.e. J_{54}). To change the position of the peak while peaking its height constant **(D)** two parameters need to be changed at the same time, the diffusion coefficient of extracellular signal and the activation of gene product 5 by gene product 4. Just changing the diffusion coefficient the position of the peaks will vary but so will their height. Simulations were run using a Forward-Euler algorithm on the Maini-Miura model for $\boldsymbol{f}$ (see S6 in SI for parameter values).

Pattern formations from homogeneous-with-noise initial patterns in H networks

As we have seen in previous section, for pattern transformations to be possible, the homogeneous steady state needs to be unstable to perturbations. This means that the gene network, $\boldsymbol{f}$ and the parameters must be such that equations (1) admit at least three equilibrium points (the initial steady state and some others equilibrium points).

Gene networks that admit only two such equilibrium points cannot lead to non-trivial pattern transformations. The reason is that the initial equilibrium point needs to be unstable for pattern transformation to occur and that noise is applied to all cells. If the initial equilibrium is indeed unstable, then noise will lead all cells to change from the initial unstable equilibrium point to the second equilibrium point. In other words, the homogeneous-with-noise initial pattern will transforms into another homogeneous pattern with just different concentrations of the gene products.

Gene networks that admit three or more equilibrium points can lead to pattern transformations. In this case, cells may move to one equilibrium point or another depending on the magnitude of the random perturbation they initially receive. For example, in the case of gene networks that admit three different equilibrium points $\boldsymbol{g}_0^*, \boldsymbol{g}^{**}, \boldsymbol{g}^{***} \in \mathbb{R}^{N_g}$ such that $g_i^{**} < g_{i0}^* < g_i^*$ for all gene product i, the gene product concentrations in each cell where the initial concentration is below g_{i0}^* may change to $\boldsymbol{g}^{**}$. Similarly, any cell where the initial concentration is above g_{i0}^* may change to $\boldsymbol{g}^{***}$. This way cells randomly settle in different equilibrium points ($\boldsymbol{g}^{**}$ and $\boldsymbol{g}^{***}$ in the example).

If the above gene networks do not include any extracellular signal, the resulting pattern will simply be a random distribution of these equilibrium points among cells. This will be, in other words, a random distribution of concentration peaks and valleys. Each of these peaks and valleys will form where the initial pattern also had, due to noise, tiny peaks and valleys. In that sense, no new concentration peaks and valleys appear and, thus, without signaling, there are no non-trivial pattern transformation (not for H networks nor in any other gene networks).

In the case of H gene networks and homogeneous-with-noise initial patterns there are, by definition, extracellular signals and, thus, concentration peaks and valleys can form where the initial pattern had tiny concentration peaks and valleys (as explained in the previous paragraph) but also at a distance from them, as explained for H gene networks under other initial patterns. In this case, thus, new peaks and valleys can arise and non-trivial patterns transformations are possible. The resulting patterns, however, still consist of random distributions of peaks and valleys (basins and ridges in higher dimensions)(see Fig.8-9). This is, in fact, what the dispersion relation indicates. Hierarchical networks are RD-unstable of the second kind and, thus, have an infinite number of unstable wavenumbers. Except for sampling limitations, the initial pattern with white noise contains all wavenumbers. This means that many, if not most, wavenumbers present in the initial noise can be amplified and, thus, the resulting patterns most likely consist of random distributions of concentration peaks and valleys (see Fig.8-9).

Emergent gene networks

L^+ gene networks

These are gene networks with one or several L^+ subnetworks (and no other types of subnetworks). From a spike initial pattern, the signal, or signals, in the L^+ subnetworks diffuse from the spike across the extracellular space and activate their own production in the cells that receive it. Since these signals promote their own production, the cells receiving them also end up producing and secreting them. As a result, each signal progressively spreads further until it ends up being expressed by all cells (see Fig.8-9). 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 (even noise is suppressed) and, thus, the only possible resulting patterns are homogeneous (i.e. no non-trivial pattern transformation occurs). The same happens for homogeneous-with-noise or combined spike-homogeneous initial patterns.

L^- gene networks

By definition, these networks only contain subnetworks with extracellular signals that inhibit their own production, directly or indirectly, in the cells that receive them. According to requirement I2, gene networks leading to non-trivial pattern transformations must include one or several positive loops (i.e. l^+) positively upstream of the gene products present in the resulting pattern. For L^- gene networks, these loops have to be intracellular, otherwise the gene network would be L^+L^- not L^-. Accordingly, we must only consider the L^- networks that include at least one L^- subnetwork downstream of at least one l^+.

Pattern transformations in L^- subnetworks from spike initial patterns

In the spike initial pattern, only the cells in the spike initially express the genes of the L^- subnetwork. Since the secreted signal inhibits itself, the expression of this signal cannot spread beyond the spike (i.e. it will only be secreted from the spike) and, thus, its peak concentration will be in the spike itself (i.e. no non-trivial pattern transformation).

Pattern transformations in L^- subnetworks from homogeneous-with-noise initial patterns

Lets us first consider the gene networks with a single L^- subnetwork and a single l^+. Among these, let us first consider the case of L^- gene networks with a single L^- subnetwork positively downstream, but not upstream, of a single l^+. Since l^+ is not affected by any extracellular signal, its gene products can only have a homogeneous resulting pattern, or a noisy resulting pattern like the one described for gene networks without extracellular signals (in the "Pattern formations from homogeneous-with-noise initial patterns in H networks" section). Neither case constitutes a non-trivial pattern transformation. By definition, the most upstream extracellular signal, *A*, inhibits itself. Then it can only be expressed in the same place than its positive regulators, l^+, and thus, these gene networks cannot lead to non-trivial pattern transformations either. The case of a L^- subnetwork upstream of an l^+ but not downstream of it does not fulfill requirement I2 and, thus, cannot lead to pattern transformations either.

The only case left is that of a L^- subnetwork that is both downstream and upstream of the l^+. In the simplest case, l^+ activates *A* and *A* inhibits some gene product in l^+, and thus indirectly all of them and itself. We call these networks over-Turing gene networks since, as we will see, they are capable of producing periodic patterns that resemble those of classical Turing gene networks (see Fig. 8-9). Since the only positive loop is intracellular, these gene networks are, depending on the parameters, either RD-unstable of the second kind or RD-stable (see Fig.6C-D) (Klika *et al.*, 2012).

In each cell, l^+ activates *A* but *A* diffuses extracellularly and inhibits l^+ in the cells that receive it and, thus indirectly itself. This means that, indirectly, the l^+ in the different cells are competing with each other. In other words, by inhibiting the l^+ of the other cells, the l^+ in a cell is indirectly activating itself. If the system is made of just two cells, the concentration of the gene products in l^+ (as well as *A*) can only increase in one cell (the one experiencing a weaker effective inhibition of *A* by l^+ at the initial time) and decrease in the other (the one subject to a stronger initial inhibition of *A* by l^+). This implies that the initial difference in the concentration of *A* between cells can only but grow until some steady state may be reached (in accordance with requirement R4). In other words, the initial concentration difference between cells (i.e. the initial noise in the pattern) becomes amplified.

The two-cell example can be used to understand larger systems with homogeneous-with-noise initial patterns. These systems can be understood as being composed of multiple overlapping pairs of cells that, due to noise, have small initial differences in the concentration of the gene products in l^+ (or any gene product upstream of it). From the arguments explained in the previous paragraph, one can expect that the initial concentration differences between each pair of cells can only but grow. In the case of systems made of many cells, however, each cell is contiguous to more than one cell (e.g., two in 1D) and, thus, it can occur that two contiguous cells both increase their

concentration of *A* because they are both contiguous to other cells that, by chance, have smaller initial concentrations of *A*. Which cells end up in concentration peaks or valleys depends on which cells had higher signal concentration in the initial pattern and the inhibition arising from the extracellular signals, as well as on the parameters of the network.

The resulting pattern consists of randomly distributed concentration peaks and valleys of *A* and the gene products in l^+ (see Fig.8-9). These patterns are very similar to the ones that can arise from H networks acting on homogeneous-with-noise initial patterns (even if the underlying dynamics are quite different). This is, in fact, what the dispersion relation indicates in the case of RD-unstable networks of the second kind: high wavenumbers are unstable and thus, they all can be amplified to form a noisy resulting pattern. Indeed, the resulting pattern can be understood, to some extent, as an amplification of the initial noise in the homogeneous-with-noise pattern (Marcon *et al*., 2016; Diego *et al*., 2018). Because of this, each resulting pattern will be different, with different peaks and valleys in different positions and different distribution of heights between them (see Fig.S3A).

Let us now consider the ensemble of over-Turing gene networks. This is any gene network combining any number of l^+ loops and L^- subnetworks downstream and upstream of each other (but no L^+ subnetwork). In all of them it is still the case, by the definition of L^- subnetworks, that extracellular signals inhibit their own production in the cells that receive them and, that thus, cells are inhibiting each others' production of each extracellular signal, directly or indirectly. This means that, as in the simple examples above, any initial difference in the initial concentration of extracellular signals between cells can only increase. In fact, over-Turing networks contain positive intracellular loops and, thus, irrespectively of their complexity, they can only be, depending on the parameters, RD-unstable of the second kind or RD-stable. In the former case, most wavenumbers would be unstable and, since there is an infinite number of them, the resulting patterns should include many of them, i.e. the resulting pattern will likely be a random distribution of peaks and valleys as in the case of the simplest over-Turing networks.

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

As in the previous initial patterns, and for the same reasons, only the over-Turing gene networks can lead to non-trivial pattern transformations. Over-Turing gene networks are known to be able to lead to relatively complex and periodic non-trivial pattern transformations from the spike-homogeneous initial pattern (Marcon *et al*., 2016; Wang *et al*., 2022). By definition, the concentration of *A* starts being higher in the spike than elsewhere. As *A* diffuses, it inhibits itself in the cells around the spike and this leads to the formation of concentration valleys of *A*. However, *A* activates gene products in l^+ and thus, similar concentration valleys of gene products in l^+ will form around the spike (see Fig.S3B-C). Since l^+ activates signal *A*, the cells in these valleys will produce less *A* and, in turn, cells beyond the valleys will receive a smaller amount of it. As a consequence, there is less inhibition of the gene products in l^+ in such cells, and the concentration of *A* and the gene products in l^+ will increase in those cells. This increase in the concentration of *A* leads to the formation of a concentration valley further away, and these valleys to further peaks, and so on. This process continues until the whole domain is occupied by a sequence of concentration peaks and valleys of *A* and the gene products in l^+. These have very similar heights, shapes and distances between them (see Fig.S3), with some variation in those closer to the spike or the boundary of the domain. In 2D there are no concentration peaks but concentric rings of high and low gene product concentration centered around the spike. In 3D there are concentric spherical shells of low and high concentration. We refer to these patterns as frozen-wave patterns as they result from the action of a patterning wavefront that moves away of the spike leaving a stationary wave-like pattern behind. 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.

The formation of these frozen-wave resulting patterns can also be understood as a consequence of the fact that, in over-Turing networks, any initial difference in the concentration of *A* between contiguous cells can only increase (as we explained in the previous subsection). In this case, the initial concentration differences are between the cells at the margin of the spike and the cells just outside of it in every radial direction. These differences can only but grow over time and thus, the concentration of *A* and gene products in l^+ will decrease in cells just outside of the spike. This, in turn, leads to a concentration difference between the cells just outside the spike and cells further away, in which the concentration of *A* and gene products in l^+ will increase again. This process continues leading to an alternation over space of concentration peaks and valleys (or concentric concentration rings and shells in higher dimensions) or, in other words, to the frozen-wave pattern.

The frozen-wave patterns cannot arise from spike initial patterns. This is because concentration valleys cannot form where gene product concentrations are zero (i.e. around the spike), since gene product concentration cannot be negative.

Changes in the parameters of over-Turing gene networks (e.g., diffusion coefficients) can lead to changes in the number, height or width of concentration peaks and valleys in the resulting pattern. However, contrary to what happens for H gene networks, concentration peaks and valleys in over-Turing networks cannot change independently from each other (see Fig.S4). This lack of variational independence in the properties of peaks and valleys is easy to understand from the fact that over-Turing gene networks 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 can happen in H gene networks). This implies that the ensemble of pattern transformations attainable by these networks is less diverse than the ensemble of pattern transformations attainable by H gene networks. In other words, the over-Turing gene networks can produce patterns with equally sized and shaped peaks, while the H networks can also produce patterns with concentration peaks and valleys with different characteristics (different heights and shapes, Salazar-Ciudad *et al*., 2000, 2001).

Gene networks combining different classes of subnetworks

Subnetworks can be combined in parallel, in series, or both. By parallel combinations we mean that different subnetworks are upstream of the same set of gene products (while being downstream of some gene product in the initial pattern, requirement I1). Since in this case the different subnetworks do not regulate each other, the resulting patterns in downstream gene products can have concentration peaks and valleys wherever their upstream gene products have them (just as described for H gene networks above, see Fig.10).

By in series combinations we mean that some subnetworks are upstream of others (i.e., the signal of the first subnetwork is upstream of the signal of the other). In this case we are interested in the resulting patterns of the downstream subnetwork (since the pattern transformations in the upstream subnetworks are unaffected by the downstream gene network and have already been described in previous sections).

Subnetworks can be combined in series positively or negatively (i.e., the signal of the upstream subnetwork is negatively upstream of the signal of the other subnetwork). In the case of negative regulation, however, some gene products in the initial pattern must positively regulate the downstream subnetworks independently of the upstream subnetwork, otherwise the genes in the downstream subnetwork only receive negative regulation and, thus, cannot be expressed (i.e. requirement I1). In this case, the resulting pattern of the downstream network would be the one it would produce on its own, but with that of the other subnetwork subtracted from it, as we described for H subnetworks (see Fig. 10). The case of positive in series combinations of subnetworks is described in the next section. Naturally, several subnetworks can be combined in series to form a chain in which each subnetwork is upstream of the next one.

Subnetworks can also be combined in series but forming a loop (i.e., the signal of each subnetwork is upstream and downstream of the signals of other subnetworks). However, if an H subnetwork is combined forming a loop with any other class of subnetwork, then the signal of the H subnetwork is indirectly regulating itself and is, thus, an emergent subnetwork. In this sense, combining subnetworks in a loop leads to emergent subnetworks.

Pattern transformations in the combination of H and L^+ and H and L^- subnetworks

As we have seen, all gene networks capable of pattern transformation can be RD-unstable of the first kind or RD-unstable of the second kind. Thus, regardless of how sub-networks are combined, there are only two main types of resulting patterns. H and L^+ subnetworks can only be RD-stable or RD-unstable of the second kind (since the only positive loop is extracellular). Beyond that we can acquire a more detailed intuition of the possible pattern transformations through some simple considerations on the pattern transformations we have described for the H and L^+ subnetworks individually.

Gene networks composed of L^+ subnetworks upstream of H subnetworks are equivalent to H subnetworks acting on a homogeneous initial pattern without noise. This is because, as explained in the L^+ section, these subnetworks lead to homogeneous resulting patterns whichever the initial pattern and, in the process, eliminate noise. Thus, the downstream H subnetworks effectively receive and input pattern that is homogeneous (i.e. without noise) and, thus, cannot lead to pattern transformations.

In gene networks in which H subnetworks are upstream of L^+ subnetworks no pattern transformations are possible downstream of the H subnetworks because the L^+ subnetworks homogenize any input patterns that the H subnetworks may convey.

Gene networks composed of L^- subnetworks upstream of H subnetworks can only lead to non-trivial pattern transformations in the case the L^- subnetworks are over-Turing, for the reasons explained in the L^- subnetworks section. The same applies to gene networks composed of H subnetworks upstream of L^- subnetworks. In this case the H subnetwork can be seen as providing the L^- subnetworks with a pattern but as we have seen L^- subnetworks cannot lead to non-trivial pattern transformations unless they are over-Turing, regardless of the initial pattern they act on.

In the case of H subnetworks upstream of over-Turing subnetworks and combined spike-homogeneous initial patterns, the frozen-wave pattern that would normally spread from the spike perturbation can spread, instead, from the peaks and valleys produced by the H subnetwork. Thus, the resulting is still a frozen-wave pattern but locally modified by the H subnetwork so that the peaks close to the initial spike can vary in size (this effect keeping radial symmetry around the spike) (see Fig.12). If the over-Turing subnetworks are upstream of H subnetworks, then the radially symmetric patterns that are attainable by the latter can be repeated around each concentration peak of the amplified noise pattern. When acting on a homogeneous-with-noise initial pattern, the only possible non-trivial pattern transformations are the noisy resulting patterns reported for both over-Turing and H networks (since both kinds of networks produce similar patterns on their own anyway).

Pattern transformations in the combination of L^+ and L^- subnetworks

In this section we first consider combinations between single L^+ and single L^- subnetworks. If an L^+ subnetwork is upstream of an L^- subnetwork, and not downstream of it (i.e., they form no loop), the L^+ subnetwork is unaffected by the L^- subnetwork and consequently, its genes will be homogeneously expressed regardless of the initial pattern (see Fig. S5-6). This reduces to what we have seen is possible for L^- gene networks acting on homogeneous-with-noise initial patterns, except that the L^+ subnetwork can eliminate the noise and preclude any pattern transformation. If the L^- subnetwork is upstream of the L^+ subnetwork, then the resulting pattern of the latter subnetwork can only be homogeneous because, as we explained in previous subsections, L^+ subnetworks produce these patterns whenever any of its signals is expressed in at least one cell (see Fig S5-6).

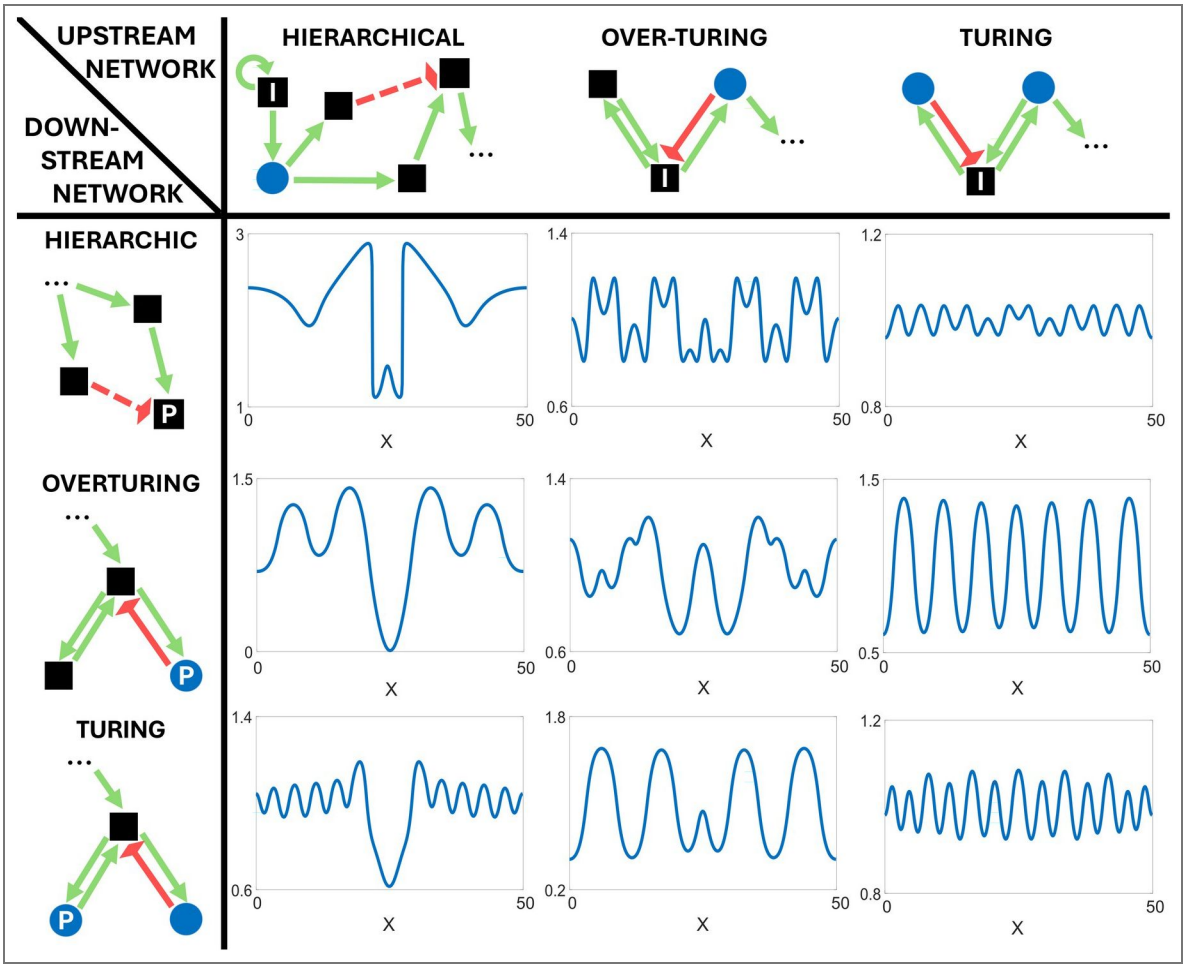


Figure 12. The combinations of hierarchical, over-Turing and Turing networks are and their resulting patterns.

The first row depicts simple examples of each class of gene network topology acting as the upstream subnetwork of the combination in series. The first column shows simple examples of each the same gene network topologies acting as the downstream subnetwork of an in series combination. Intermediate panels show each type of possible resulting pattern arising from a spike-homogeneous initial pattern from each combination. Note that all pattern transformations are non-trivial. Network colors and shapes as in Figure 2. Dashed arrows represent the different, and strictly non-linear, regulations in the H^0 networks. *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 for $\boldsymbol{f}$ (see S6 in SI for parameter values).

L^+ and L^- subnetworks can be combined into loops. Previous research has extensively studied pattern transformations occurring from such networks (Cotterell & Sharpe, 2010), for the case of gene networks with up to three gene products. From these, the only resulting patterns and developmental dynamics that we have not discussed so far are those of Turing gene networks, also called Turing mechanisms (Turing, 1952; Murray, 2002; Maini *et al*., 2006; Meinhardt, 2008). These occur, for example, when a L^+ subnetwork is positively upstream of an L^- subnetwork, and the latter is negatively upstream of the former (e.g., the signal of the latter inhibits, directly or indirectly, the signal of the former).

In Turing's seminal work (Turing, 1952), each subnetwork is in fact a single extracellular signal, but it has been widely reported that these mechanisms also apply to gene networks where extracellular signals regulate each other indirectly, through an intracellular part of the gene network (Salazar-Ciudad, *et al*. 2000; Satnoianu, *et al*., 2000; Maini *et al*., 2006; Meinhardt, 2008; Cotterell & Sharpe, 2010).

Given that Turing gene networks contain an extracellular positive loop (i.e. an L^+ subnetwork), they are, depending on the parameters, either RD-stable or RD-unstable of the first kind (see Fig. 6A-B). In the former case the resulting patterns are periodic. For homogeneous-with-noise initial patterns, a good approximation for the wavenumber of the resulting pattern is given by the unstable wavenumber closest to the maximum of the real part of the principal branch of the dispersion relation (Murray, 2002) (see Fig. 6A-B). For combined spike-homogeneous initial pattern a more detailed analysis is required to understand the wavenumbers in the resulting pattern (Tarumi & Mueller, 1989; Klika *et al*., 2024) but the resulting pattern is also periodic.

In Turing gene networks, the larger wavenumbers are stable (i.e. do not grow) because of the L^+ subnetwork. The signal in the L^+ subnetwork diffuses between cells and activates its own production in nearby cells. This produces a homogenizing effect in which, contrarily to what happens in over-Turing networks, the initial differences in concentration between contiguous cells do not necessarily grow over time. In fact, the positive extracellular loop precludes large concentration differences between nearby cells and thus, precludes large unstable wavenumbers (i.e. having many concentrations peaks per unit of space and so, large relative differences in concentration between contiguous cells).

In 1D, the possible resulting patterns of Turing networks are similar to the periodic patterns arising from over-Turing gene networks (see Fig. 8). The difference is that, while in Turing networks these periodic patterns can arise from both homogeneous-with-noise and combined spike-homogeneous initial patterns, in over-Turing networks they can only arise from combined spike-homogeneous initial patterns. In 2D and 3D, Turing networks acting on homogeneous-with-noise initial patterns can lead to resulting patterns consisting of several concentration peaks (e.g., dots) or ridges (e.g., stripes) with a constant spacing and regularly distributed over space, or forming labyrinths (Meinhardt, 1982; Murray, 2002), while over-Turing networks acting on those initial patterns can only produce noisy resulting patterns (see Fig.9). From combined spike-homogeneous initial patterns in 2D and 3D domains, over-Turing networks can lead to resulting patterns consisting of concentrically arranged concentration ridges and basins due to the radial symmetry of the problem (see Fig.9). This is also possible from Turing gene networks (Meinhardt, 1982).

As in the case of over-Turing gene networks, changes in the parameters of a Turing gene network (e.g., in diffusion coefficients) can change the global number, height or width of concentration peaks and valleys in the resulting pattern (Turing, 1952; Murray, 2002; Maini *et al*., 2006; Meinhardt, 2008). However, in contrast to what we see in H networks (Salazar-Ciudad et a., 2000), these parameter changes lead to all concentration peaks and valleys to change in the same way. The reasons for this difference are the same we discussed for over-Turing gene networks (see Fig.S4 and S5) (i.e. a relatively small number of gene products is responsible for the resulting pattern and no part of the network is responsible for any specific subset of concentration peaks or valleys). For this same reason the ensemble of distinct resulting patterns attainable by Turing gene networks is smaller and less diverse than the ensemble of distinct resulting patterns attainable by H gene networks. Simply, the ensemble of possible Turing

networks can only produce resulting patterns where all concentration peaks (and valleys) are identical while the ensemble of possible H networks can produce resulting patterns where different concentrations peaks and valleys can have different heights, weights and spacings (see Salazar-Ciudad, *et al*. 2000, 2001 for a more detailed exploration).

Turing subnetworks can be combined into composite gene networks (see Fig.12). 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). As long as these combinations the only positive loops are extracellular, the system can only be, depending on the parameters, RD-stable or RD-unstable of the first kind (just like single Turing networks). Consequently, regardless of how complex the composite network is, the possible resulting patterns are still periodic patterns, that may combine several wavelengths (Fujita & Kawaguchi, 2013; Moustakas-Verho *et al*., 2014). Combining Turing gene networks with over-Turing gene networks in series leads to composite gene networks that behave either as RD-unstable networks of the first or second kind depending of model parameters (see section S2 in SI).

In general, gene networks that combine Turing gene networks and H gene networks in series have variational properties with features of both the Turing and H gene networks (see Fig.12). There are, in fact, many articles studying this (Salazar-Ciudad *et al*., 2001; Miura, 2013; Green & Sharpe, 2015; Glim *et al*., 2021; Tzika *et al*., 2023). If the Turing networks are upstream of the H networks, then each concentration peak resulting from the Turing network acts as a different spike in the input pattern received by the H network. Thus, the peaks and valleys resulting from the H network can be repeated around each of the concentration peaks produced by the Turing network. If the peaks produced by the H network are wider than those produced by the Turing, however, these latter peaks may fuse and the resulting pattern simply resembles that of the Turing network (see Fig.12).

In gene networks in which H networks are upstream of the Turing networks, the resulting pattern can be periodic, but the H networks can make the periodic pattern slightly different in different spatial regions (by specific gene product in the H networks regulating specific gene products in the Turing networks). Thus, the height or spacing of concentration peaks in the resulting pattern of the Turing network can be larger, or smaller, where patterned genes of the H network are expressed (see Fig.12).

Besides being combined into Turing or over-Turing subnetworks, or their combinations, L^+ and L^- can be combined into any arrangement (e.g. into multiple nested loops). As we have shown in previous sections, however, a gene network with such complex combinations can only be, nonetheless, RD-unstable of the first or second kind. In the former case the resulting pattern will be periodic, as when combining Turing gene networks. In the second case, we do not know for sure which are the possible resulting patterns but since the number of unstable wavenumbers is infinite, we can expect that, from homogeneous patterns with noise, the resulting patterns are amplified noise patterns (just as combining several over-Turing networks). Similarly, from combined spike-homogeneous patterns we expect froze-wave patterns, possibly combining different periods.

Discussion

The main conclusions of this article are that all gene networks capable of non-trivial pattern transformations can be classified into three distinct topological classes (and their combinations) and that each of these classes allow for qualitatively different types of resulting patterns. For gene networks within each class we found some additional topological requirements.

All gene networks can be decomposed into subnetworks. Because we study pattern transformation by extracellular signaling, we decompose gene networks based on the subnetworks downstream of each of its extracellular signal. These subnetworks are classified based on whether their extracellular signals are positively downstream of themselves (L^+ subnetworks), negatively

downstream of themselves (L^- subnetworks) or not downstream of themselves (H subnetworks). This classification exhaust all possibilities, except for subnetworks that arise as a combination of several of these simpler subnetworks.

We have shown that pattern transformation requires gene networks with positive regulatory loops and that, if these loops are extracellular, then the resulting patterns, when heterogeneous, are periodic. We have also shown that, if the positive regulatory loops are intracellular, then the resulting patterns, when heterogeneous, are characterized by an infinite amount of unstable wavenumbers. However, negative interactions are also required for pattern transformation. The combination of the former findings with this latter requirement implies that there are only three types of gene networks leading to pattern transformations: 1) Hierarchical gene networks with positive intracellular loops and negative regulations 2) Over-Turing gene networks (i.e. gene networks with negative extracellular loops and positive intracellular loops forming a loop between them) 3) and Turing gene networks (i.e. gene networks with positive and negative extracellular loops forming a loop between themselves). These three fundamental classes, and their combinations, exhaust all possibilities for gene networks capable of non-trivial pattern transformations through extracellular signaling.

In this article we also show that these three classes of gene networks, and their combinations, each lead to specific types of resulting patterns (see Fig.8-9). Hierarchical networks lead to resulting patterns that are radially symmetric and made of any combination of concentration peaks and valleys (ridges and basins in higher dimensions). Depending on the parameters of the network, these resulting patterns can contain more or less peaks and valleys with different positions, heights and different shapes. In over-Turing gene networks infinitely-many wavenumbers are linearly unstable and thus, many get amplified in the resulting pattern. From homogeneous-with-noise initial patterns, this leads to amplified-noise patterns, while from combined spike-homogeneous patterns, this leads to radially symmetric periodic patterns extending all over the domain. Similarly, we also prove that Turing networks only amplify a finite number of wavenumbers, if any, and then, the only non-trivial pattern transformations they can lead to result in periodic patterns. In the last part of the article, we discuss how the further combination of hierarchical with over-Turing, hierarchical and Turing or Turing and over-Turing networks leads to resulting patterns that can be seen, to a large extent, as the combination of the types of patterns possible from each of the three classes of networks.

Throughout our article, we have considered that cells are arranged in simple regular ways over space. In our arguments, the only element that depends on the geometry of the cellular arrangement is the explicit form of the eigenmodes in (6), whereas the construction of the dispersion relation itself is geometry-independent. The existence of a limited number of topological classes capable of generating patterns does not depend on the geometry of space (i.e., on how cells are distributed within 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 the specific resulting patterns possible from each specific gene network. Thus, for example, the height, spacing and shape of concentration peaks and valleys can change close to the boundaries of the system (Diambra & Costa, 2006; Glimm *et al*., 2014; Castelino *et al*., 2020). In Turing networks it is even possible that the resulting patterns changes substantially depending on the shape of the system (e.g., from producing stripes to producing spots), but in all cases the type of pattern, in the coarse way we have defined, remains periodic (Murray, 2002).

All the gene network topologies capable of pattern transformations that we report 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 non-trivial pattern transformation, given the biologically reasonable restrictions on $\boldsymbol{f}$ that we describe. We think this is important both for theory and empirical studies. For theory, it is important because we provide a general description of what is possible at the level of non-trivial pattern transformations and underlying gene network topologies (as long as there is no cell movement). Experimentally, this is also important because it

provides a simple guideline for experimental developmental biologists trying to understand pattern transformations in specific organs over a set of developmental stages. Thus, if no cell movements occur during these stages, the underlying gene network should have a network topology and variational properties among the ones described in this article (see Fig.13). This significantly narrows down the range of possible underlying gene networks and patterning mechanisms to consider when trying to understand the development of a multicellular system. Similarly, the results of this article allow to improve the study of the evolution of gene networks since they show that the gene networks capable of non-trivial pattern transformations can only change within a finite set of classes (Salazar-Ciudad *et al*., 2001).

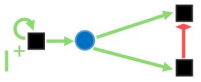
TYPE OF NETWORK	DO FINAL PATTERNS DEPEND ON INITIAL PATTERNS?	CAN SOME PEAKS VARY INDEPENDENTLY FROM EACH OTHER	DIVERSITY OF POSSIBLE RESULTING PATTERNS
Hierarchic networks 	YES ✓ H-Spike IP→ Spiked pattern. Hom. IP → Noisy pattern.	YES ✓ Upstream gene products are unaltered by changes in downstream gene products.	VERY HIGH ↑↑ A hierarchic network can be engineered to reproduce almost any sequence of peaks.
Over-Turing networks 	YES ✓ H-Spike IP→ Periodic pattern. Hom. IP → Noisy pattern.	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 ✗ H-Spike IP→ Periodic pattern. Hom. IP → Periodic pattern.	NO ✗ Changes in any gene product produce global changes in the final pattern.	LOW ↓ In 1D, only periodical patterns. In higher dimensions, spots or stripes.

Figure 13. Variational properties of the gene network topologies capable of pattern transformation.
Network colors and shapes as in Figures 2 and 4.

Of the three classes of gene network topologies we identify, two have been widely studied before: hierarchical and Turing networks. 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; 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; Gilbert and Barresi, 2023) and they are, by far, the easier to understand. This, and the fact that many networks seem hierarchical when only some of their interactions are known (Salazar-Ciudad, 2009), may have biased research to focus on them. Although over-Turing gene networks seem simpler and easier to understand than Turing networks, they have only been discovered very recently and only based on theoretical work (Cotterell *et al*., 2015; Marcon *et al*., 2016; Wang *et al*., 2022). It is thus, an open and suggestive question whether this class of gene networks 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 over-Turing networks instead, since the latter produce resulting patterns that are similar to the former, especially when the organ is effectively 1D.

Data availability

All data is provided within the article itself (this is a purely theoretical article).

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Additional files

SI

Additional information

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Peer reviews

Reviewer #1 (Public review):

Summary:

The authors tackle a long-standing question in developmental theory: given a gene-regulatory network that includes extracellular signaling, 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 organizing insight for both theorists and experimentalists.

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

Weaknesses:

(1) After the resubmission, I still have concerns regarding the formal definition of "non-trivial transformations" (P1/P2) and its application to noisy or multi-dimensional systems. The criteria rely on counting "new" critical points (maxima/minima). In their response, the authors argue that the diffusion operator instantly smooths discontinuous white noise, allowing critical points to be properly defined. However, this very smoothing process passively generates a landscape of new, smooth local extrema from the initial noise. Consequently, trivial diffusive regularization could inadvertently fulfil the criteria for a "non-trivial" transformation, leaving the definition conceptually problematic. Furthermore, when extending the framework to 2D/3D, the manuscript assumes that starting from a central "spike" will robustly preserve radial symmetry, yielding concentric rings or shells. This overlooks the fundamental nature of macroscopic mean-field models like reaction-diffusion equations. The realization of the final multidimensional pattern depends strictly on the

stability of the solution against ubiquitous perturbations (including angular modes) rather than solely on the deterministic symmetry of the initial condition. It remains unclear how the current framework accounts for spontaneous symmetry breaking in cases where these angular modes become unstable, challenging the assumption that radial symmetry will strictly dictate the outcome. We note that the authors' use of noise as an initial condition does not resolve this fundamental issue. Reaction-diffusion equations inherently describe mean-field dynamics, meaning that microscopic fluctuations are continuously present in any real system, regardless of whether explicit stochastic terms are written into the equations. Ultimately, if a symmetric mean-field solution is structurally unstable to these inherent fluctuations, it simply cannot be realized in nature.

(2) Theoretical limitations in the application of Linear Stability Analysis (LSA): I remain uncertain about the framework's reliance on LSA to categorize macroscopic transformations, especially those arising from large initial perturbations (spikes). In their rebuttal letter, the authors justify this by assuming the perturbation remains small over a short time interval. However, because the study aims to describe stationary, asymptotic states, applying a linear approximation that relies on transient t->0 conditions to predict long-term global stability is not fully resolved.

(3) In the previous round of the review, I suggested that a biomolecular sink, such as A+B -> AB reaction, could break the approach. In their response letter, the authors defend their approach by arguing that such reactions can be accommodated by their abstract constraints (R1-R5) as long as the signs of the Jacobian elements remain invariant. However, the problem I see here is not the sign of the interactions, but the severe loss of spatial homogeneity.

When a macroscopic initial perturbation (a "spike" of morphogen) is introduced into a domain with a strong bimolecular sink, it will inevitably cause massive local depletion of the consumed substrate near the source. Consequently, the background state of the system will rapidly evolve into a profile with macroscopic spatial gradients long before any spontaneous pattern-forming instability takes over. Mathematically, this dictates that the system no longer possesses a homogeneous steady state, and the Jacobian matrix becomes explicitly space-dependent, which should break the classical LSA approach.

Discussion:

The study offers a solid conceptual organization of pattern-forming networks. However, the theoretical bridge between infinitesimal linear stability and macroscopic, non-linear pattern emergence still presents some uncertainties. The way the current framework formally treats noise, multi-dimensional symmetry breaking, and large initial perturbations leaves some questions open regarding its broad analytical applicability to real biological tissues.

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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, and finally 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 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 signal and intracellular responses, corresponding to previously known mechanisms that can generate spatial patterns: Difference in morphogen concentrations in space, noise-amplification, and Turing pattern.

https://doi.org/10.7554/eLife.107563.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Public Reviews:***
>
> ***Reviewer #1 (on non-trivial pattern transformations):***
>
> *(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 slopebased 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.*

There was indeed a problem on how we defined non-trivial pattern transformations in the original version. This definition was not clear enough beyond 1D. We now provide a simple clear definition in the main text that applies to all dimensions ("P1" and "P2" in the second page of the introduction).

As we now explain through the main text, even if the solution of the heat/diffusion equation depends on the dimension of the system, our classification of gene networks (and the mathematical analyses we use) does not depend on the dimensionality of the system. However, some aspects of the specific pattern transformations possible from these networks depend on the dimensionality of the system. In the current version of the article, every time we explain something about the resulting patterns in 1D, we also explain it for the resulting patterns in 2D and 3D. We also have added figures for the 2D cases (in current Fig.1 and Fig.9). We now explicitly explain how the possible resulting patterns in space can depend on the boundaries and shapes of the system (i.e. the distribution of cells in space) (see specially the 5th paragraph of the discussion).

The criticisms about "slope-based measures" mentioned by reviewer 2, is now addressed in a paragraph at the end of the introduction (here we added it):

"It is worth noting that these three basic initial patterns correspond to spatially discontinuous functions: in homogeneous with noise initial patterns, white noise is discontinuous by definition; in spike and combined spike-homogeneous initial patterns, there is a concentration discontinuity between cells on the edge of the spike and nearby cells outside

the spike. However, once extracellular signal diffusion begins, these sharp boundaries are smoothed into differentiable gradients, where critical points can be properly defined (e.g., at the center of the initial spike)."

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 (on linearization):***
>
> *(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 (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.*

We used three linearizations in the original version of the manuscript. One was to analyze hierarchic networks (in the Hierarchic networks section). In the new version of the article we do not use any linearization to study the hierarchic networks, so this problem is solved.

The second linearization was in section S3 on transitive chains. We realized that this section is not really necessary at all for the article so we deleted it.

We keep the third linearization but we now explain why such linearization is useful and valid in a section called "Linear stability analysis". Thus, through this section we justify this choice (explicitly in its two first paragraphs).

Regarding Reviewer 2 concerns about large perturbations, we acknowledge that the phrasing using "arbitrary height" may have been confusing. As we now explain in the linear stability analysis section, linear stability analysis assumes perturbations to be small.

For the homogeneous-with-noise initial pattern, as we explain, these perturbations are assumed to be small because they are actually molecular noise.

For the spike initial pattern and hierarchic networks the perturbation is not necessarily small. However, by the definition of the spike and combined homogeneous-spike initial patterns, all cells outside the spike start with the same concentration of the extracellular signals that are secreted from the spike (e.g. zero). Thus, even in the case in which

extracellular signals concentrations in the spike would be unrealistically high, the amount of extracellular signal diffusing from it can be considered small by simply considering it at a small enough time interval. Thus, right outside the spike the diffusion of extracellular signals from the spike can be treated as a continuous small perturbation for which one can study the stability, as we do in the "Linear stability analysis section". This we now explain at the end of the introduction and in the "Linear stability analysis" section when we talk about the initial patterns again.

In the following, we respond to the remaining concerns raised by the reviewers:

> ***Reviewer #1 (Public review):***
>
> *(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 original 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. We were wrong. We have now moved crucial parts of the supplementary to the main text and adapted the rest of the text accordingly. The most important of those is the new "Linear stability analysis" section and the associated dispersion relation (e.g. Fig.6).

> ***Reviewer #2 (Public review):***
>
> *(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 are actually simulating the full nonlinear system described by Equation (1). In the current version we are more explicit about this. As we describe in the introduction and, now, through all the text several times (e.g. in the last paragraph of the model section and in the paragraph before the linear stability section), the aim of the article is to describe necessary requirements for non-trivial pattern transformations. We did not intent to describe all necessary requirements nor sufficient requirements. These requirements are at the level of gene network topology not at the level of f or its parameters. In other words, we just claim that gene networks having specific topological features can lead to some specific types of non-trivial pattern transformations but not to others. We do not say for which specific fs (or its parameters) these pattern transformations are possible, we just say that this can happen for some f, as long as these fulfill our requirements. We do show, however, that without some specific topological requirements there are non-trivial pattern transformations that are not possible, no matter the f (this explicitly stated in the last paragraph of the model section and in the paragraph before the linear stability section). Thus, all the simulations shown in the figures are just examples, with specific fs, of the types of non-trivial pattern transformations possible from each type of gene network topology.

In all simulations we used the f of the Maini-Miura model. We could have chosen other ones but we happen to chose that f. The presentation of the Maini-Miura model has been revised to improve clarity (equation S6.1 in SI). This model we are simulating fully, we are not doing any linearization for the simulations. That may not have been explained clearly enough in the previous version of the article. We just happen to make a change of variable that may have been confused as a linearization. In the current version, the existence of a homogeneous steady state is parameterized by a tunable $\mathbf{g}^*$, that can be chosen as $\dot{g}_i^* = 0$ for spike initial patterns or g for noise-homogeneous and spike-homogeneous initial patterns. We have also included a proof that the model equations satisfy our conditions R1-5. Indeed, the model is non-linear as long as $\sigma_i \neq 0$ for some gene product (as we explicitly assume).

> *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.*

In the revised manuscript, homogeneous steady states are parameterized by a tunable $\mathbf{g}^*$, which can be chosen as $\dot{g}_i^* = 0$ for spike initial patterns or g for noise-homogeneous and spike-homogeneous initial pattern. Function **f(g)** in (S6.1), as well as the specific non-linear entries used in certain simulations, are constructed such that $\mathbf{g}^*$ is indeed a steady state of the system and that conditions R1-R5 are satisfied. We have also corrected some typos in section S6 (previously section S8) of the Supplementary Information, that we believe may have induced the confusion indicated by this reviewer.

> *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.*

As described above we just chose an example f to exemplify the non-trivial pattern transformations possible from each class of gene network topologies. There is no special reason to include, or exclude for that matter, cubic cross-species interactions since the point is just to exemplify the types of possible pattern transformations from each type of gene network topology.

In addition, we believe that part of the reviewer's concern may have arisen from a notational ambiguity in the previous version of the manuscript, which has now been corrected: the matrix appearing in **f(g)** has been renamed from **J** to $\mathbf{W}^\mathbf{T}$. As stated in the main text, the jacobian of the regulation function **f(g)** evaluated at the homogeneous steady state must coincide with the transpose of the network weight matrix. With the current equations (S6.1), we have $\boldsymbol{J}_f(\boldsymbol{g}) = \boldsymbol{W}^T - 3\boldsymbol{S}(\boldsymbol{g} - \boldsymbol{g}^*)^2$, from which we easily get $\boldsymbol{J}_f(\boldsymbol{g}) = \boldsymbol{W}^T - 3\boldsymbol{S}(\boldsymbol{g} - \boldsymbol{g}^*)^2$. Also, it is clear that the Jacobian of **f(g)** is not independent of **g**.

> *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.*

The Heaviside function does not preclude inhibition, it precludes gene product concentration to be negative. In the current version of the article we do not use the Heaviside function but another similar, but continuous, function. Having this function can indeed affect the dynamics but: 1) does not violate our requirements on f 2) Does not affect which non-trivial pattern transformations are possible from which gene network topology. Without this function non-trivial pattern transformations are still possible from the spike initial pattern through hierarchical networks, in the way we describe in the article. The Heaviside function (and the one we now use) simply allows that to happen more easily, i.e. for a larger range of parameter values. With this function large inhibitions do not lead to negative gene products concentrations while without it, this can happen for some parameter combinations. None of the arguments nor proves in our article requires the Heaviside, or any similar function. Again this is simply because our aim is to identify topological requirements that are necessary, but not sufficient, for non-trivial pattern transformation. So an f that leads to negative gene products concentrations for some parameter combinations but to non-trivial pattern transformations for others, is still valid example of our points (although not the most interesting or realistic example f).

We distinguish between the spike and combined spike-homogeneous initial patterns simply because they are biologically quite different, i.e. in the former the gene product in the spike is only expressed in the spike and nowhere else. As we describe in the current version the pattern transformations possible from these two different initial patterns are very similar. In the same way, which gene network topologies can lead to which types of non-trivial pattern transformations is not affected by using the Heaviside functions or not (although this can affect the range of parameter values in which this happens).

> *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.*

The implementation of nonlinear entries in **f(g)** whenever they are needed is now made explicit in the corresponding subsection in the main text and in section S6 in the Supplementary Information. This entries also satisfy conditions R1-R5 around the steady state given by $\mathbf{g}^*$. Again we should insist that the simulated fs are nonlinear (as now explicitly explained in the SI).

> *(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:*
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> *"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).*

The result of which gene network topologies can lead to pattern transformations are based on a linear stability analysis and some logical arguments. As we now explain through the text none of them depends on the number of dimensions nor on the shape of the arrangement of cells. The geometry of the domain can influence the specific form of the resulting patterns, but it does not alter the broader type of resulting patterns (e.g., periodic patterns, peaks emerging around a spike, etc.) that a given gene network topology can produce. We now explicitly discuss these dependencies in the 5th paragraph of the discussion.

> *"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).*

We now provide a more detailed justification of this statement and the limits of its applicability. This is now in section: “The ensemble of possible pattern transformations from spike initial patterns in H networks“. To make this section easier to understand, however, we have also done changes through all the hierarchic networks sections.

> *"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 (at least far away from the boundary). 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 ridge surrounding the spike in two dimensions, and each ridge in two dimensions becomes a spherical ridge shell around the spike in three dimensions. In the current version we explain what happens in 1D but also, in the same places, what happens in 2D and 3D (and we have added figures to visualize this in 2D, e.g. Fig.1 and Fig.9)).

> *(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 may have been too brief. We now provide a more detailed description in the section “Gene networks combining different classes of subnetworks” and in its sub-sections. There we explore the different ways in which signal subnetworks can be combined (upstream, downstream, in series, in parallel, etc.). However, this section cannot be understood (and that may have been the problem in the original version of the manuscript) without the linear stability analysis section that is now in the main text, and the associated discussion on the dispersion relation and results related to it. These are important because they apply to all gene networks and, thus, constrain the possible gene network topologies and the types of possible pattern

transformations. In other words, whichever ways gene networks are combined, they will always be RD-stable (i.e. no pattern transformation) or RD-unstable of the first (periodic resulting patterns) or second kind (other patterns we discuss). In the current version, we combine this fact with other arguments to describe the types of pattern transformations possible by gene networks combining the different classes of subnetworks.

> *(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.*

We now clarify all these points in the first three paragraphs of the “Methods: the Model” section. We have also included a figure for that clarification (Fig.3).

> ***Recommendations for the authors:***
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> ***Reviewer #1 (Recommendations for the authors):***
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> *I suggest the following changes for each weakness I mentioned in the Public Review:*
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> *(1) Presentation*
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> *(R1.1) (a) Add a one-page "Key Requirements" table (e.g., immediately after the Model section) that lists every requirement code (R1-R5, I1-I2, RH1-RH2, etc.), its one-line statement, and the SI section where it is proved.*

In the new version of the article each requirement has its own paragraph starting with the requirement label, e.g. R1 (in bold): ….. We introduce each requirement there where they are justified or proven, otherwise the reader may not know where do they come from. We have also hyperlinked all requirements and most equations so that the reader can easily go back to the explanation of each requirement and equation.

> *(R.1.2) Provide more figures illustrating the general structure of networks when you describe them; the network sketches could be folded into a single summary figure, so the reader sees all motifs at once. For example, in lines 304-311, it took me a while to understand if the requirement means just A -> k - ... ⊣ j, or it additionally requires A->...->j (through another pathway). It seems that the full requirement is A → k ⊣ j together with an independent positive route A → j. A figure describing the network structure, or at least a schematic "inline" plot in the spirit of what I just wrote, could help. This is just one example, but the text consists of a constant flow of such "diagrams encrypted in prose".*

We have followed the reviewer’s suggestions. Not all fit in a single figure so we have constructed new figures 4 and 5 for that purpose.

> *(R.1.3) (b) Also consider supporting the main text with some key formulas and arguments from SI. My overall suggestion here is that it would be great to make the main text less prosaic and more self-consistent, if the journal requirements allow it.*

After the suggestions by both reviewers, and for the sake of clarity, we have actually moved (and clarified) several key parts of the SI into the main text. These include the whole “Linear stability analysis” and “Positive regulatory loops determine the kind of RD-instability” sections. These parts, although quite mathematical, facilitate the understanding of our results.

> *(2) Linearisation*

> *(R.1.5) It's clear that keeping non-linearity is complicated and maybe redundant, but please, discuss the assumption of linearity explicitly, especially in the scope of relevance for the real systems, and explain why it's not important, if so. I guess that relaxing this assumption may affect the argumentation in many places, for example, equation (3) of the main text could break (i.e., if the signaling molecule can be consumed in some reaction of A+B->AB kind).*

We agree that the original version was not explicit enough about the reasons for the linear approximation. The first and last paragraphs of the section “Linear stability analysis” are explicitly devoted to justify this linearization. Moreover, the hierarchical network section is now written without using the linearization.

We are not sure we understand which is the problem with the A+B→AB reaction. We are not assuming any specific f function, just the ensemble of functions that fulfill our requirements (R1 to R5). It is only for the simulations that we have to use a specific f. The reactions suggested by the reviewer could represent an f of the form d[AB]/dt=fAB([A]*[B])-m*[AB]**n for AB and d[A]/dt=-fAB([AB]) and d[B]/dt=-fAB([AB]), where fA and fB are functions that decrease with their arguments. We see no reason why there cannot be a fAB that fulfills our requirements. For example fAB=[A]*[B]/(K+[A]*[B])-m*[AB]. See also related comments in the public comments file.

> *(R.1.6) Please, provide a separate section where you reformulate the definition of "non-trivial pattern transformation" for two- and three-dimensional domains, and summarize in this section why the analysis provided for 1D is relevant for higher-dimensional systems. By now, I'm not convinced.*

There was indeed a problem with the way we described non-triviality beyond 1D in the original version of the article. We have now refined the definition of pattern transformations so that it is understandable in 2D and 3D. This definition is presented in the introduction already (in P1 and P2). We have modified figure 1 accordingly.

> ***Reviewer #2 (Recommendations for the authors):***
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> *Major Issues*
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> *(1) Mathematical Proofs*
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> *(R2.1) We strongly recommend that the authors revisit the mathematical derivations or provide a clear and rigorous justification for the assumptions made therein. These assumptions currently appear unjustified or overly simplistic, especially in light of the nonlinear dynamics the authors aim to describe. The authors should comment on why they expect their results to generalize to all complex network structures, as claimed, and not only apply to the simplified examples analyzed in the paper.*

The article has now been restructured to that end. Concerning the assumptions, they are now all explicitly described in the “Methods: the model” section. Concerning the derivations they are through all the results section. A major change in this line has been the moving of part of the supplementary into specific sections in the main text (and the consequent adaptation of the rest of the text). There are important points of the derivation that may have been buried into the old supplementary and that are crucial to understand the whole argument in the article. In fact, a large part of the results section is just a long argument to show that there are essentially only three classes of gene network topologies that can lead to non-trivial pattern transformations. These arguments are summed up in the last paragraph of the new section “Positive regulatory loops determine the kind of RD-instability” and in the first paragraph of the discussion. In brief:

(1) Pattern transformation requires gene networks with extracellular signals

(2) Applying previous mathematical results we show (given the broad requirements on f we have) that pattern transformation is only possible in gene networks that contain positive regulatory loops.

(3) Applying previous mathematical results we show that in the gene networks in which these loops are extracellular, the only possible non-trivial pattern transformations lead to periodic resulting patterns.

(4) Applying previous mathematical results we show that in the gene networks in which these loops are INTRAcellular, the only possible non-trivial pattern transformations do not necessarily lead to periodic resulting patterns.

(5) Using simple logical arguments we also show that no non-trivial pattern transformations are possible in gene networks without negative interactions.

(6) All the above points combined shows that there are only three classes of gene networks capable of nontrivial pattern transformations. 1) Those with intracellular positive loops, extracellular signals that do not affect themselves and some negative regulation by those (that we call hierarchic networks) 2) Those with intracellular positive loops and extracellular signals that affect themselves negatively (that we now call over-Turing networks) 3) Those with extracellular positive loops and an extracellular negative loops (that following previous work by others are called Turing networks).

(7) Following previous research and different developmental arguments we explore the types of patterns transformations each of these three classes of gene networks can lead to. These types are characterized only in broad and potential terms. We say nothing about the parameters values for which any gene network leads to any specific pattern transformation. What we say is which types of pattern transformation may be possible (for some possible parameter combination) and which ones are not possible from gene network topology alone (based on the types of loops and so on).

> *(R.2.3) Additional to the examples provided in the Public Review, claims such as "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" (l. 34ff)*

This statement was originally intended as an introduction of the text following after it but it seems now clear that this was not apparent enough. This statement has been deleted but we convey a similar message letter in the text, now once its justification is provided. In fact, the justification for this statement is the summary we just described in the previous point (R.2.2) and it is discussed over the main text and summarized in the last paragraph of section “Positive regulatory loops determine the kind of RD instability”.

> *(R.2.4) and "The same applies to the topologies we found not to be able to lead to non-trivial pattern transformation" (S7) are not or inadequately justified and should be either substantiated or significantly toned down.*

The same comments that above apply.

> *(R.2.5) (a) We advise the authors to argue why it is enough to prove key results by considering linear dynamics (see S2-S7). While linearization is a common technique, the authors themselves emphasize the importance of nonlinearities in pattern formation throughout the paper.*

In the current version we provide an explicit justification for this in the section “Linear stability analysis”, especially in its first paragraph. Moreover, for the analysis of the hierarchical networks we do longer use any linearization.

> *(R.2.6) (b) To make linear analysis meaningful, we suggest restricting the initial conditions to small fluctuations (e.g., small spikes or noise), which would justify using linearization to investigate the onset of non-trivial pattern formation. Alternatively, the authors should attempt to generalize the results to fully nonlinear dynamics, ideally for a broader class of functions f.*

As we now explain, the homogeneous-with-noise initial pattern already correspond to small perturbations around the homogeneous steady state (due to molecular noise). In addition, for the spike and spike–homogeneous initial pattern we now explicitly consider spikes of small amplitude. We acknowledge that the use of larger spikes in the previous version could lead to misunderstandings regarding the validity of the linear approximation, even though it does not contradict the assumptions underlying the analysis. In these initial patterns, pattern formation arises because the signal secreted from the spike diffuses into the surrounding domain, so that cells outside the spike experience only small deviations from the equilibrium concentration.

Larger spikes may induce stronger deviations in cells located very close to the spike; however, because the spike occupies a region that is very small relative to the total domain size, these local effects do not influence pattern formation in the bulk of the domain. A similar situation occurs with boundary effects in cells located near the domain limits, which likewise do not affect the pattern formation process away from the boundaries. We have clarified this point in the revised manuscript, both in the final sentences of the Introduction and in the description of the initial conditions in the fourth paragraph of the “Linear stability analysis” section, where we explicitly state that each initial pattern can be interpreted as a perturbation of an otherwise homogeneous pattern.

> *(R.2.7) (c) The assumptions required for the proofs should be explicitly stated and justified. At present, the logic behind the chosen constraints on f is unclear, and the flow of the argument suffers as a result.*

The actual justification for the requirements (i.e. constraints) on f are biological (and we now explain them more explicitly when we introduce these requirements). Most of the mathematical proofs do not require these requirements except when we explicitly say so.

> *(R.2.8) (d) The illustrative functions provided in some of the proofs in the SI (e.g. S5.2.1 "To see this, let us consider, for example, that they are both quadratic monomials of the form f_k(g_A)=B_k g_A^2 and f_j(g_A)=B_j g_A^2") do not satisfy the authors' own stated conditions (e.g., this function violates requirement R4 (l.197 f)). More suitable examples should be selected to ensure consistency between assumptions and illustrations.*
>
> *We have changed the whole section (based on the comment R.2.9 from the same reviewer). We now provide arguments in the main text that generally do not rely on specific fs.*
>
> *(R.2.9) (e) Currently, all mathematical results are confined to the appendix. We recommend including key insights from the proofs in the main text to improve readability and to allow the main claims to stand on their own. For example, the section on the requirements RH2a and RH2b (l. 320 - l. 335)) would benefit strongly from the insights from S5.2.1*

We agree. We have moved the linear stability analysis and the dispersion relation section to the main text. We have also moved what used to be S5.2.1.

> *(2) Simulations*
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> *The simulations raise, as mentioned in the Public Review, several concerns regarding their generality and validity.*
>
> *(R.2.10) (a) We recommend validating the simulation results by comparing them with simulations of the full nonlinear equations. The authors should at least provide the equations for the full dynamics and explain how the expansion is performed and why it is valid. This also includes verifying the assumed steady states (g_i=0 and g_i=c_i, where 1/c_i = \mu_i or \hat{\mu}_i).*

We are simulating the whole non-linear equations. Here it is important to stress, as we do now in the main text, that our results apply to any f, as long as it fulfills our R1-R5 requirements. However, for the simulations in the figures we have to use a specific f (since there is an infinite amount of fs that fulfill our requirements). Again the figures are just examples to visualize the types of resulting patterns and gene networks we talk about.

In the original version we may not have been clear enough about the equations used for the simulations. The presentation of the Maini-Miura model has been revised to improve clarity (equation S6.1 in SI). In particular, the existence of a homogeneous steady state is now parameterized by a tunable $\mathbf{g}^*$, that can be chosen as $\dot{g}_i^* = 0$ for spike initial patterns or $\dot{g}_i^* = 0$ for homogeneous-with-noise and spikehomogeneous initial patterns). We have also included a proof that the model equations satisfies our conditions R1-5. Indeed, the model is non-linear as long as $\sigma^i \neq 0$ for some gene product (as we explicitly assume).

The derivation of this cubic model from a separate expansion of general reaction-diffusion dynamics can be found in the original paper (Miura & Maini, 2004), with further applications to pattern formation that supporting its validity in subsequent works (Marcon et al., 2016; Diego et al., 2018). Importantly, this expansion is independent of the linearization performed in the main text of our article to derive the dispersion relation. The reference to this separate expansion in the previous version was included solely for contextual purposes; however, we have removed it in the revised manuscript to avoid potential confusion.

> *(R.2.11) (b) The use of a Jacobian that is independent of the steady-state contradicts the assumption of nonlinearity (requirement R2 (l. 192f)) of f. We ask the authors to clarify this.*

We believe this concern arises from a notational ambiguity in the previous version of the manuscript, which has now been corrected: the matrix appearing in the regulatory term has been renamed from $\mathbf{J}$ to $\mathbf{W^T}$. As stated in the main text, the jacobian of the regulation function $\mathbf{f(g)}$ evaluated at the homogeneous steady state must coincide with the transpose of the network weight matrix. With the current equations (S6.1), we have $\boldsymbol{J}_f(\boldsymbol{g}) = \boldsymbol{W}^T - 3\boldsymbol{S}(\boldsymbol{g} - \boldsymbol{g}^*)^2$, from which we easily get $\boldsymbol{J}_f(\boldsymbol{g}) = \boldsymbol{W}^T - 3\boldsymbol{S}(\boldsymbol{g} - \boldsymbol{g}^*)^2$. Also, it is clear that the Jacobian of $\mathbf{f(g)}$ is not independent of $\mathbf{g}$.

> *(R.2.12) (c) In Figure S3 and similar simulations, the implementation of the nonlinear terms is ambiguous. The function f shown does not correspond to the Jacobian, and it remains unclear how these components are ultimately implemented in the simulation code. Additionally, as mentioned, it does not fulfill the necessary conditions for the global steady state.*

The implementation of nonlinear entries in $\mathbf{f(g)}$ whenever they are needed is now made explicit in the corresponding subsection of section S6 in the SI. With the new notation it becomes clearer that the fs used can fulfill the necessary conditions for the global steady state.

> *(R.2.13) (d) The given function f_8 in S8.10.2 cannot correspond to the mentioned network since the number of gene products does not match the Jacobian and the network.*

This was a typo that has now been corrected.

> *(R.2.14) (e) The given parameters for the figures in the SI do not match the figures. Please check and ensure that the correct figure is referenced (e.g., S8.2 Figure 3)*

This was a typo in the numeration of the subsections in the SI that has now been corrected.

> *(R.2.15) (f) It is unclear which units are used, and the units used for the non-dimensionalization should be provided so one can relate them to biological systems.*

It is now explicitly stated in the revised version that the model equations are formulated in arbitrary units. This implies that the model dynamics are consistent with the characteristic units of any particular biological system under consideration. No non-dimensionalization of the model equations has been considered.

> *(3) Conceptual and Structural Clarity*

The manuscript suffers from a lack of structural clarity, which affects both readability and scientific coherence.

> *(R.2.16) (a) In one of the central figures (Figure 4) supporting their main claim, the naming of the network is not consistent with the main text. The network category referred to as "Over-Turing" is never mentioned in the main text. We suspect this should actually be labeled as the "noise-amplifying network."*

Indeed. This has now been corrected. We now use only the term “Over-Turing” in the article.

> *(R.2.17) (b) The Supplementary Information includes an analysis of dispersion relations to classify patternforming networks, but this approach is not mentioned or referenced in the main text.*

This part of the SI has been moved to the main text and the dispersion relation has been fully and explicitly integrated in the overall argument of the article.

> *(R.2.18) (c) In relation to Figure 6, we found that the concept of "diversity of possible final patterns" would benefit from a clearer definition and explanation. It is not immediately evident how this diversity is measured or what criteria are used to compare different networks. For instance, it is unclear why the Over-Turing network - which generates both periodic and noisy patterns - is considered to exhibit low diversity, whereas the Turing networks, which produce only periodic patterns, are described as having high diversity.*

This was just a large typo. The figure has been corrected. The reasons for this differences are now described in the last three paragraphs of the section “The ensemble of possible pattern transformations from H gene networks and spike initial conditions” for the hierarchical networks and in the last paragraph of the section “Pattern transformations in L- subnetworks from spike-homogeneous initial patterns ”, for the noise amplifying networks and in the seventh paragraph of the section “Pattern transformations in the combination of L+ and L- subnetworks” for the Turing networks.

> *(R.2.19) (d) Additionally, the dependence of final patterns on initial conditions is not clearly described. It seems that this relationship is only analyzed for non-trivial pattern formations, but this is not explicitly stated. Clarifying these points in the caption of Figure 6 would greatly help readers understand the interpretation and significance of the results presented in this figure.*

Indeed, we have done nothing for the trivial pattern transformations. We are now more explicit about this already from the introduction. This article is only concerned with non-trivial pattern transformations. For each type of gene network we now provide a more detailed description of how the resulting pattern depends on the initial pattern (in the sections for each gene network).

> *(R.2.20) (e) The significance statement is simply a verbatim repetition of parts of the abstract. This defeats its purpose, which is to articulate the broader implications of the work. We urge the authors to rewrite this section with a focus on significance rather than summary.*

We have now corrected this.

> *(R.2.21) (f) We suggest including a dedicated figure to illustrate the biological model, depicting cells, intracellular and extracellular compartments, and the presence or absence of boundaries between adjacent cells. Such a figure would significantly enhance readers' understanding of the system being discussed.*

We have now done that. See new figure 3.

> *(R.2.22) (g) We encourage the authors to strengthen the 2D and 3D results presented in the paper by adding supporting citations, sharing implementation details, or providing a more in-depth analysis of these systems. If such additions are not feasible, it may be best to remove references to the 2D and 3D systems to maintain clarity and focus.*

In the new version of the article we explain why our results on which gene networks can lead to pattern transformation do not depend on the dimensionality of the system. In fact, none of our proofs or arguments assumes or requires a specific number of dimensions. The networks are the same no matter the number of dimensions. The types of possible patterns can be seen as manifesting themselves differently depending on the number of dimensions. In the current version of the manuscript we explain now, every time we explain a resulting pattern, how the pattern is in 1, 2 and 3 dimensions and why. We have added Figures 1 and 9 for that purpose. As we explain in the text, the resulting patterns that are noisy would be noisy no matter the number of dimensions and the ones that are based on a spike in the initial pattern have necessarily radial symmetry (in any number of dimensions). Similarly the periodic patterns will be periodic no matter the number of dimensions (although some aspects of it will change). Similarly, in the 5th paragraph of the discussion we discuss the effects of the shape of the system and the boundary. There was a problem with the definition of pattern transformation we used, but this has now been corrected, in P1 and P2 in the introduction.

> *(R.2.23) (h) The results section lacks a consistent structure. Section titles do not clearly indicate which phenomena or initial conditions are being analyzed, making it hard for readers to track the logical progression of the study.*

Now the results start with some introductory results with the subsections:

“Basic requirements on gene networks capable of pattern transformation”

The rest of the results are split into four clearly differentiated sections:

“Gene network classification”

“Linear stability Analysis”

“Positive regulatory loops determine the kind of RD-instability”

“Hierarchical Networks”

"Emergent networks".

"Gene networks combining different classes of subnetworks"

The last three sections have several sub-sections inside.

We think that the titles of the sections are self-explanatory since hierarchical networks contain only H subnetworks while the emergent networks contain L+ or L- subnetworks and the last major sections is about how all these can be combined.

> *Minor Issues*
>
> *(1) Notation and Terminology*
>
> *(R.2.24) (a) Variable naming is inconsistent throughout the paper. Terms like g_A(x) and A(x) (S5.2.1) are used for gene network concentrations without consistent usage. The naming of genes in networks also varies between the main text, SI, and figures. I.e., sometimes genes are labelled with small, sometimes with large letters, and sometimes with numbers.*

This has now been corrected.

> *(R.2.25) (b) It would improve clarity to use distinct notations for intracellular vs. extracellular concentrations and gene expressions. Ensure networks and examples are consistent across all figures, captions, and supplementary materials. For example, RH2a and RH2b have different networks in the main text compared to the SI.*

As we now explain in the third paragraph of the "Methods: the model" section we consider, for simplicity, that gene products are either intracellular or extracellular. In that sense there is no possible ambiguity. As explained in that section, again for simplicity, we do not consider the receptor nor the signal transduction pathways of signals. This means that an extracellular gene product can "directly" regulate intracellular gene products. Because of that, we think that using different notations for extracellular and intracellular gene products would make things more confusing. We have corrected the misnaming between main text and figures.

> *(R.2.26) (c) We suggest using distinct notation for the gene product itself and for its small deviation from a homogeneous steady state in the SI. This would help clarify whether specific statements apply only within the linearized regime or can be generalized to the full nonlinear dynamics.*

We do that in the new version of the article.

> *(R.2.27) (d) Line 327 contains a mistake: g_k = g_j should be expressed as a proportional relationship. The division by g_A also seems unnecessary - please revise.*

This is now explained in a different way so this mistake does not apply.

> *(2) Model Description*
>
> *(R.2.28) (a) Justify why boundary effects and spatial separation between cells can be neglected in the model.*

This is now discussed in the 5th paragraph of the model section. We do not claim that boundary effects are negligible. We claim, instead, that which are the gene networks that can lead to pattern transformations do not depend on the boundaries. The same occurs for the types of resulting patterns, in the coarse way we use, possible from each gene network and initial pattern.

As stated in the first two paragraphs of the model section, the spatial separation between cells can be ignored because we assume there are many cells in the system and these are evenly spaced and sized (at least roughly). That is usually the case in animal development, although not always (there are exceptions in the very early stages of many marine invertebrates), and we do not claim to know exactly what happens in those cases: as we stated in the first paragraph of the introduction we assume systems made of many small cells.

> *(R.2.29) (b) State explicitly that only extracellular gene products are assumed to diffuse - this is currently only mentioned in the SI.*

This is now explicitly stated early on in the first three paragraphs of the model section and also after the introduction of the model equations (1)-(3).

> *(R.2.30) (c) In the Supplementary Information, the authors state that both extracellular and intracellular gene products can exhibit non-zero diffusion, which appears inconsistent with the conceptual framework and probably is a typographical error.*

This was indeed a typographical error. It is now corrected.

> *(3) Assumptions and Requirements on f*
>
> *(R.2.31) (a) The equation for requirement R5 is incorrect as written in the main text and should be reformulated more rigorously. The condition should be stated for all constant values of g_i (and g_j) to avoid misinterpretation; otherwise, one might assume all matrix elements must have the same sign.*

This has now been corrected.

> *(R.2.31) (b) Clarify what restrictions on f prevent pathological nonlinearities like 1/(g_k + \epsilon), which would contradict the assumed behavior at high concentrations.*

We do not understand this criticism. 1/(g_+\epsilon) fulfills our requirements on f and we do not see how is that pathological. We are unsure of what the reviewer means by the assumed behavior at high concentrations.

> *(4) Figures and Captions*
>
> *(R.2.32) In Figure S3b, the diagram shows gene 5 being activated by gene 4, yet the caption states this is a negative regulation - please correct.*

This has now been corrected.

> *(5) Readability and Formatting*
>
> *(R.2.33) (a) Improve navigation by hyperlinking references to equations, figures, and requirements throughout the document.*

In the new version we have inserted these hyperlinks.

> *(R.2.34) (b) Adding hyperlinks to the requirements would additionally help the reader to keep track of them*

In the new version we have inserted these hyperlinks.

> *(We.2.35) (c) Correct inconsistent or mismatched equation numbers and references. E.g. SI S5.1 is not referring to the correct equation (the equation it should be referring to would be Equation 3), and the reference to Figure 7 in part of the dispersion relation is wrong (as far as we see, this should be Figure 5).*

This has all been corrected now.

> *(R.2.36) (d) Clarify ambiguous language in the introduction. For instance, the description of spike patterns (lines 136f) as a single cell spike contradicts the stated width (SI) and the visual representation involving 500 cells from the figures.*

This has now been corrected.

> *(R.2.36) (e) The discussion of 2D and 3D simulations appears limited to the "noise amplifying" network. It's unclear whether a similar analysis was done for other network types.*

In Figures 1 and 9 and through the text we discuss all types of patterns in 2D and 3D.

> *(6) Typos*
>
> *(R.2.37) Typos in the text (The following is just a small selection of the typos we came across. Since there are quite a few throughout the manuscript, we may not have caught all of them. We kindly recommend that the authors carefully proofread the full text to ensure consistency and clarity):*

We have corrected all the indicated typos and proofread the whole manuscript and SI.

> ***Reviewer #3 (Recommendations for the authors):***
>
> *Major concern:*
>
> *(R.3.1) Pattern formation can be induced by the positional information, and reaction-diffusion/Turing mechanisms is a foundational idea in the field. As in the references the manuscript cited, these paradigms were already clearly articulated and synthesized (e.g., Green & Sharpe's work (2015)). Moreover, the search for minimal network topologies that can generate Turing patterns has been extensively explored in Zheng et al. (2016). The novelty of the present work is unclear. It might offer a fresh perspective on an established problem, but it does not seem to present fundamentally new biological or mathematical advances.*
>
> *If the authors wish to strengthen the novelty and impact of the manuscript, they should consider explicitly acknowledging prior work and positioning their contribution as a formal extension or generalization, not discovery. To enhance the practical relevance of their work, the authors could demonstrate how their framework can be used to predict or classify gene network behaviors in pattern formation that are not easily identifiable through experimental approaches alone. For example, they could show how their classification helps distinguish between Turing, hierarchical, and noise-amplifying dynamics in complex or ambiguous biological systems, thereby offering a guiding tool for experimental design or interpretation.*

Indeed, the gene networks we identify have been identified before. We were and we are quite explicit about it, in the discussion, and we do cite the relevant work on that (including the one suggested by the reviewer). The novelty of the work is not identifying these gene networks, nor minimal ones, but showing that these are all the possible ones for pattern transformation (that there is no new type of network), this has not been done before (not even intended) and we are very explicit about that being our results (first paragraphs of the discussion).

> *Minor concern:*
>
> *The writing style and language usage can be improved for clarity. Some explanations in the results and discussion can benefit from tight editing to eliminate redundancy and*

> *improve readability.*

We have corrected all the indicated typos and proofread the whole manuscript and SI.

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