Different Cell Fates from Cell-Cell Interactions: Core Architectures of Two-Cell Bistable Networks

Hervé Rouault[†‡] and Vincent Hakim[†*]
[†]Laboratoire de Physique Statistique, CNRS, Université P. et M. Curie, École Normale Supérieure, Paris, France; and [‡]Institut Pasteur, Paris, France

ABSTRACT The acquisition of different fates by cells that are initially in the same state is central to development. Here, we investigate the possible structures of bistable genetic networks that can allow two identical cells to acquire different fates through cell-cell interactions. Cell-autonomous bistable networks have been previously sampled using an evolutionary algorithm. We extend this evolutionary procedure to take into account interactions between cells. We obtain a variety of simple bistable networks that we classify into major subtypes. Some have long been proposed in the context of lateral inhibition through the Notch-Delta pathway, some have been more recently considered and others appear to be new and based on mechanisms not previously considered. The results highlight the role of posttranscriptional interactions and particularly of protein complexation and sequestration, which can replace cooperativity in transcriptional interactions. Some bistable networks are entirely based on posttranscriptional interactions and the simplest of these is found to lead, upon a single parameter change, to oscillations in the two cells with opposite phases. We provide qualitative explanations as well as mathematical analyses of the dynamical behaviors of various created networks. The results should help to identify and understand genetic structures implicated in cell-cell interactions and differentiation.

INTRODUCTION

How regulatory interactions between genes, mRNAs, and proteins determine distinct cell fates is a central question of developmental biology. In a number of cases, cell-cell interactions play an important role in allowing neighboring cells to adopt different fates. The well-studied Notch-Delta pathway (1) provides several biological examples of this process, ranging from gonadogenesis (2) and vulval development (3) in *Caenorhabditis elegans* or sensory organ development (4) in *Drosophila melanogaster* to neurogenesis in vertebrates (5). In addition to experimental studies, different theoretical approaches have been followed to better understand the structure and interaction requirements of a cell fate specifying network, from general mathematical analysis of simple model networks to detailed studies of specific systems.

Mathematical studies have, for instance, served to emphasize the interest and potential role of network bistability in cell fate specification (6). Similarly, spontaneous symmetry breaking between two cells has been analyzed in a minimal model of lateral inhibition (7). More detailed models of lateral inhibition have been developed in the context of specific biological examples (8,9). Despite their interest, both approaches have limitations. In a reduced mathematical description an effective interaction can reflect different underlying biophysical mechanisms. This is an advantage in terms of generality but also a source of difficulty when one wishes to identify a particular mechanism in a given network of biophysical interactions. A further limitation resides in the choice of the simplified model itself, which usually does leave many possibilities unexplored. Even if less apparent, this is also true to some extent with detailed modeling because choices have to be made for many parameters, or for the details of many interactions, for which only scarce experimental guidance exists in most cases.

It therefore appears worth sampling and characterizing, with minimal a priori bias, core network structures that produce a given dynamical behavior. An exhaustive search for networks that perform a given task (10) is feasible only by restricting oneself to very small networks and a limited set of interactions. An alternative goal-oriented computer-assisted procedure (11–20) consists of producing computer models of genetic networks, in an iterative way, under the guidance of a score function to be optimized. This type of evolutionary search has produced interesting networks that in a number of cases resemble known biological networks or, at least, appear to capture some of their essential structures. For instance, evolutionary simulations performed to create single-cell oscillators (13) have highlighted the so-called mixed-feedback loop in which a protein both transcriptionally regulates a gene and directly interacts with the protein it produces (21,22). It is a simplified but quite recognizable version of the central mechanism used by circadian clocks in different organisms and it appears to lie at the core of several other biological oscillators as well (23). Similarly, for cell-autonomous patterning networks in a static gradient, evolutionary simulations produced chains of transcriptional repressors. The simplest instance is an incoherent feed-forward loop that serves to produce localized gene expression at an intermediate value

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*Correspondence: hakim@lps.ens.fr

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of a graded signal (15,18) and of which many biological examples are known. Recently, evolution of cell-autonomous patterning networks has further served to try and shed light on the properties of Hox genes (20).

In this work, we use an evolutionary algorithm to investigate the possible architectures of networks that are able to create different cell fates in two adjacent cells. We first consider basic network motifs that are able to drive single cells toward two different fates in a cell-autonomous manner. The obtained bistable motifs were studied in previous works, which highlighted the role of protein sequestration in a complex (13,22), as we recall. We then show that it is possible to extend the evolutionary procedure to include interactions between neighboring cells. We obtain different network architectures that rely on different mechanisms to produce two exclusive cell fates in a pair of interacting cells. Some networks simply provide precise models of commonly considered mechanisms. Others highlight little-noted or new mechanisms that rely on protein-protein interaction, in a crucial way. They should help to recognize these structures or similar ones in actual biological networks. The produced networks are also useful as working examples of two-cell bistable networks. They show, for instance, that cell interactions allow network designs that more reliably produce two distinct cell fates when initial conditions vary.

Mathematical analyses of several interesting obtained network structures are provided in the Supporting Material, section 2. A complete description of each discussed network is provided in the Supporting Material, section 3.

METHODS

Algorithm overview

We follow an evolutionary methodology that was previously proposed (13) to generate networks of interacting genes and proteins that achieve a prespecified function in a single cell. The algorithm was already adapted to evolve networks creating patterns of gene expression in a linear array of noninteracting cells (15). Here, we further extend the procedure to produce networks that allow for direct cell-to-cell interactions.

The algorithm used in this work differs from the algorithm of (13) at several points detailed in the Supporting Material, section 1. Both algorithms evolve a collection of genetic networks using repeated rounds of growth, mutation, and selection. Each genetic network in the collection consists of a set of genes and associated mRNAs and proteins, interacting via transcriptional interactions as well as posttranscriptional interactions. The dynamics of each network is described by a set of ordinary differential equations.

Posttranscriptional interactions can be of three types, "dimerization, "phosphorylation", and "activated phosphorylation". Dimerization consists in introducing a complexation reaction between two proteins A and B to produce the complex AB as described by the chemical reaction, $A + B \rightarrow AB$. Phosphorylation of protein A consists in the production of a modified form of protein A, $A \rightarrow A^*$. Activated phosphorylation is similar to phosphorylation but the reaction depends on a protein B that is part of the described network, $A + B \rightarrow A^* + B$.

In each of these posttranscriptional reactions, the new produced protein (i.e., AB or A^*) is considered as a new protein. Its properties are independent of the properties of the other reactants. No regulation of mRNA translation by RNA is considered in the present algorithm.

To illustrate how evolutionary simulations proceed, two typical runs for the creation of cell-autonomous bistable networks are displayed in Fig. S4.1.

RESULTS

We investigate the network motifs that produce different stable expression profiles in two cells with identical genetic networks. The chosen evolutionary score function favors dynamics in which two cells with different initial concentrations of a protein A end up in different well-separated states, as described in the Supporting Material, section 1.5.

Cell-autonomous bistable networks

Before analyzing the role of interactions between cells, we consider networks that function in a cell-autonomous way. In this case, the difference in initial protein concentration produces different persistent states in the two cells, only when the network is bistable. The possible structure of such networks is a long-pondered question (24), which has previously been addressed with the help of evolutionary simulations (13). Posttranscriptional interactions between proteins are found to play an important role in the produced networks, as noted previously (13) and described below.

The two most frequently produced networks are depicted in Fig. 1, A and B. In these simple two-gene networks, bistability is achieved by combining a single transcriptional activation with the formation of a protein complex that sequesters a transcriptional regulator.

In the first one, displayed in Fig. 1 A, which we term the autoactivation and complexation (AAC) network, protein A transcriptionally activates its own gene and can also bind to protein B in a transcriptionally inactive complex. The AAC is bistable in a large window of parameters as shown in Fig. 1 A. One stable state is obtained when the concentration of protein A is sufficiently high to bypass complexation with B and allow a high concentration of free A to transcriptionally activate gene a. On the contrary, in the low A state, the few A proteins produced are sequestered in complexes with B and cannot activate gene a. It should be noted that complexation removes the need for cooperative autoactivation of gene a. A mathematical analysis of the AAC network dynamics is provided in the Supporting Material, section 2.1.

The second frequently obtained bistable network is displayed in Fig. 1 B. In this so-called mixed-feedback-loop (MFL) (21,22), autoactivation is replaced by a transcriptional repression of gene b by protein A. As in the AAC network the complex between proteins A and B is transcriptionally inactive. The network is bistable when the unrepressed production rate of protein B is larger than the production rate of protein A, as precisely shown in Fig. 1 B. When the concentration of B proteins is low, it cannot prevent transcriptional repression of gene b by A and a stable low B state

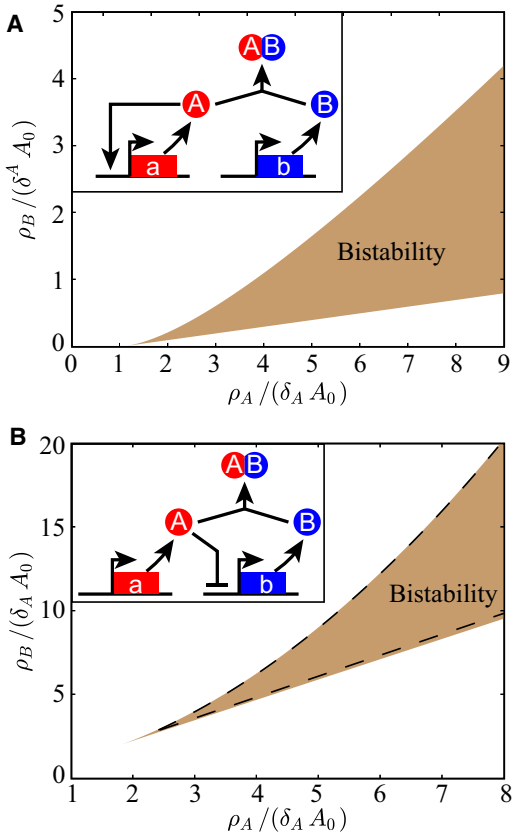

FIGURE 1 Simple bistable networks that give two different stable states of gene expression. (*A*) AAC network (network depicted in the figure inset).The bistable region of the AAC network is shown in the parameter plane of dimensionless protein production rates $(\rho_A/(\delta_A A_0), \rho_B/(\delta_A A_0))$ (see the Supporting Material, section 2.1). (*B*) MFL (network depicted in the figure inset). The bistable region of the MFL is shown in the dimensionless protein production rate $(\rho_A/(\delta_A A_0), \rho_B/(\delta_A A_0))$ parameter plane. Note that in the MFL, complexation can be replaced by a catalytic modification of protein *A* by protein *B*. This is also the case for the AAC network when saturation of the catalyst is taken into account, as explained in the Supporting Material, section 2.

ensues. On the contrary, in the high *B* state, transcriptional repression is prevented by efficient sequestration of *A* in complexes with *B*. Mathematical results for the bistable MFL (22) are summarized in the Supporting Material, section 2.

When posttranscriptional interactions were allowed in evolutionary runs, purely transcriptional networks were rarely produced. To create a significant number of purely transcriptional networks, we removed posttranscriptional interactions from the set of possible interactions, in a subset of simulations.

With cooperative transcriptional regulation, bistability was achieved by the familiar autoactivation of a single gene, either directly or indirectly, for instance via cross-inhibition of two genes, as shown in Fig. S4.2, *A* and *B*.

Perhaps more surprisingly, purely transcriptional bistable networks were also created when transcriptional activation was chosen to be noncooperative. A two-gene network of this kind that was repeatedly produced in our simulations is shown in Fig. S4.2 *C*. An effective sigmoidal activation of gene *a* by A results from the fact that different concentrations of *A* are required for half-activation of a direct and an indirect self-activation loops, as explained in the Supporting Material, section 2.

The core bistable networks described in this section and the mechanisms on which they are based play an important role in the two-cell networks investigated in the following sections.

Cell-cell interaction and exclusive cell fates

We have adopted the simple and generic formulation depicted in Fig. 2 *A* and detailed in the Supporting Material, section 1.4 to describe a direct interaction between cell 1 and cell 2. It first requires the choice of two proteins, for example *A* and *B*, which mediate the interaction. The action of the signal-sending cell on the signal-receiving cell is then represented by the transformation of *B* into a modified B^* in the signal-receiving cell when *A* is present in the signal sending-cell. The process is meant to model in a simple way the cleavages of the Notch receptor protein and release of Notch intracellular fragment upon interaction with its ligand Delta (25,26). N-cadherin (27) and protocadherins (28,29) are also thought to mediate intercellular signaling by releasing soluble intracellular fragments that can enter the nucleus. Alternatively, our simple description could also represent the activation of a receptor tyrosine kinase upon binding of its ligand. An example of this latter type in cell patterning is for instance provided by the interaction between R8 and R7 photoreceptor cells in the developing *Drosophila* eye, mediated by the binding of Boss to the receptor tyrosine kinase receptor Sevenless (30).

The results of including interactions between cells in the algorithm were quantified by performing 1000 evolutionary simulation runs of 1200 generations each (Fig. 2). Networks that successfully performed the task were produced in more than half (627/1000) of the runs. The distribution of the pruned networks as a function of the number of proteins they use is shown in Fig. 2 *B*. To check that this was not dependent of the maximum allowed numbers of proteins and transcriptional regulations, another set of simulations was performed in which these numbers were doubled. Very similar results were obtained as shown in Fig. S4.3. We proceed to describe the different types of produced networks.

The simplest case: interaction between homologous proteins in the signal-sending and signal-receiving cells

When interactions were included, some evolutionary runs (91/627) still led to the cell-autonomous networks described

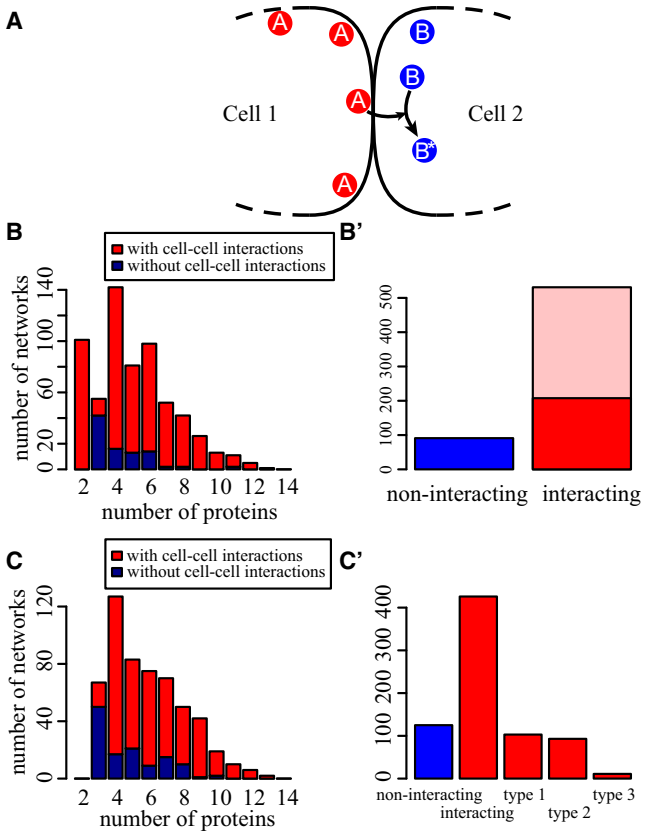

FIGURE 2 Interaction between two cells, description and statistics of the produced networks. (*A*) Schematic representation of the chosen interaction model between two cells. Protein *A* in the signal-sending cell (cell 1) induces a transformation in the signal-receiving cell (cell 2) of protein *B* into protein *B**. Of course, the reciprocal interaction between *A* in cell 2 and *B* in cell 1 also exists but is not pictured for clarity. See the Supporting Material, section 1.4 for the explicit mathematical description. (*B*) Numbers of created networks successfully producing two different fates in neighboring cells in 1000 evolutionary runs in which *A* and *B* can be identical in a signaling couple (*A, B*). The networks are sorted by the number of different proteins they use (a protein and its modified form(s), e.g., *B*, *B** and *AB* are all considered different so that, for instance, the AAC and MFL switches appear as three protein networks). (*B′*) Numbers of created networks that are either cell-autonomous (*noninteracting*) or that use homologous interaction with a signaling couple (*A, A*) (*plain*) or signaling between heterologous couples only (*light*) in the same simulation runs as in B. (*C*) Identical to (*B*) but when A and B in a signaling couple are required to be heterologous. (*C′*) Numbers of created networks that are either cell-autonomous (*noninteracting*) or use cell-interactions (*interacting*). The numbers of interacting networks of the three different types with <6 proteins and a single signaling pair are also shown (see main text for details).

in the previous section. However, most created networks (536/627) made use of interactions between the two cells, as shown in Fig. 2 *B′*. Without additional constraints (see below), in ~40% of the successful runs (205/536), the algorithm chose the signal-sending protein, *A*, and the signal receiving protein, *B*, to be identical. Moreover, about half (101/205) of these networks consisted of only two proteins, *A* and its modified form A^* and coincided with the simple network displayed in Fig. 3 *A*. In this case, *A* simply activates its own gene and transforms *A* into A^* in the neighboring cell. Therefore, when the concentration A_1 of *A* is high in cell 1, autoactivation of *A* is prevented in cell 2 and the concentration A_2 of *A* is cell 2 is low. In turn, this makes action of A_2 on A_1 weak and as a result inactivation is not prevented in cell 1. Quite remarkably, the network is bistable in a two-cell context even when autoactivation is not cooperative and therefore the network is not bistable in a single cell context. This two-cell network functions on a principle that is very similar to the AAC single-cell network with the two different proteins of the AAC network replaced by homologous proteins in different cells. Mathematical details of the analysis of this simple network are provided in the Supporting Material, section 2.

In the bistable regime, the symmetry between the two cells is spontaneously broken: the state in which the two cells have identical expression profile is unstable and even a small difference between the initial concentrations of *A* in the two cells is sufficient to send one cell toward one fate and the other cell toward the other fate. On the contrary,

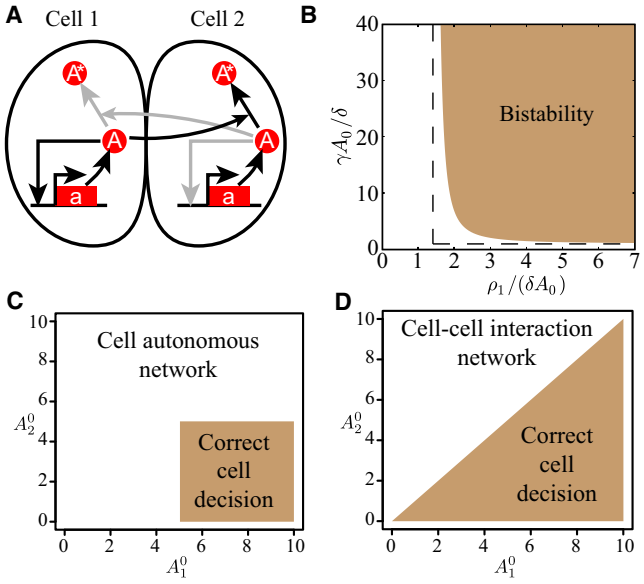

FIGURE 3 Simplest network of two interacting cells with exclusive fates: interaction between homologous proteins in the signal-sending and signal-receiving cells. (*A*) Schematic explanation of the network dynamics in two cells that are in different states. Here and in the following pictures, gray arrows denote nonactive interactions whenever cell 1 is in the high A state. (*B*) Phase diagram of the two-cell network showing the parameter domain in the (ρ_1, γ)-plane (ρ_1: protein production rate, γ: interaction strength) for which bistability exists. In this parameter domain, the symmetry between the two cells is spontaneously broken and the two cells settle in different states. The precise definition of the parameters as well as the equations of the network are provided in the Supporting Material, section 2.4. (*C*) Sketch of the domain of initial *A* concentrations in which the first cell assumes a high *A* fate and the second one a low *A* fate for a cell-autonomous bistable switch. The chosen threshold of 5 for the high *A* state has been chosen arbitrarily. (*D*) Same as *C*, for a bistable network based on cell-cell interactions as the network depicted in *A*.

for cell-autonomous bistable networks, a threshold in the initial concentration of A delimits the basins of attraction of the two states. One fate or the other is produced depending on whether the initial concentration of A is above or below the threshold. This makes the interacting network more robust than cell-autonomous networks to initial concentration variations as depicted in Fig. 3, *C* and *D*.

We inspected the homologous interaction networks with >2 proteins. Although we did not precisely quantify it, a large proportion of these more complicated networks appeared to use the same mechanism as the prototypic network of Fig. 3, although decorated with further interactions. For example, in many networks the self-activation of gene a proceeded indirectly via the activation of an activator of a.

Networks with interaction between heterologous proteins in the signal-sending and signal-receiving cells are of three general types

In some of the previously described simulations, protein A in the signal-sending side and protein B on the signal-receiving side were different. To further investigate this biologically interesting case, we conducted an additional 1000 evolutionary runs in which it was enforced that an interacting A-B pair was made of two different proteins. About half (551/1000) of the runs produced successful networks. Most of the produced networks (426/551) made use of cell interactions (Fig. 2 *C*). The most commonly created bistable networks were of three different types (Fig. 2), which are displayed in Figs. 4–6 and detailed below. We systematically examined networks with six proteins or less (255/426). A fraction of these (48/255) made use of two signaling couples and were not further analyzed. In the remaining ones (207/255), type 1 and type 2 networks were the most frequently created, in comparable proportion (respectively 103/207 and 93/207), whereas type 3 was produced less frequently (11/207).

We defined type 1 networks as the networks in which B^*, the modified signaling protein, only interacted with other proteins. These dominantly (56/103) consisted in the simplest network, depicted in Fig. 4 *A*. Quite strikingly, this network does not make use of any transcriptional regulation. It is simply based on complexation between A and B^*, the modified B protein. The presence of A in cell 1 transforms B into B^* in cell 2. B^* then binds to A in cell 2. Therefore, the level of free A is low in cell 2 and cell 2 cannot signal back to cell 1. As a result, the level of A and nonactivated B are high in cell 1, whereas B is activated in cell 2 (and the level of A low). Of course, an opposite stable state is possible with the roles of cell 1 and cell 2 reversed. This network is bistable and produces two cells in different states. Notably however, bistability is only possible in a multicellular context. Dissociated single cells are monostable. Bistability is achieved for this simple network in a large parameter range when the degradation rate of protein

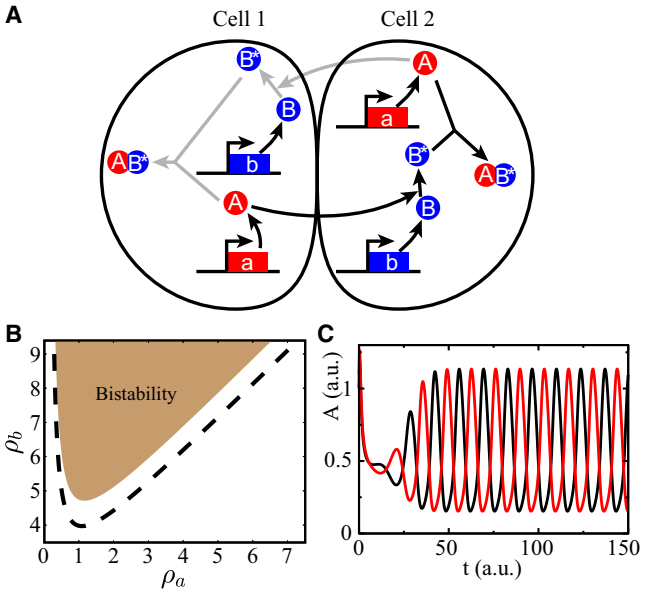

FIGURE 4 Two interacting cells with exclusive fates: type 1 network with interaction between heterologous proteins in the signal-sending and signal-receiving cells. (*A*) Schematic explanation of the network dynamic in two cells that are in different states. (*B*) Network phase diagram. The different dynamical regimes in the protein production rates (ρ_a, ρ_b)-parameter plane are shown in a case when the degradation rate of A is smaller than the degradation rate of B, $\delta_A = 1 < \delta_B = 2$. The region where the two-cell network is bistable and the two cells assume different states is shown. The dashed line is the approximate expression derived in the Supporting Material, section 2.4.2 (Eq. 2.56) for the bistability boundary, that is valid for rapid complexation between A and B^*. See the Supporting Material, section 3 for the network equations and precise definitions of the parameters. (*C*) Dynamical traces showing oscillations in the two-cell network (one curve for each cell) in different states when the degradation rate of B is smaller than the degradation rate of A ($\delta_A = 1 > \delta_B = 0.25$).

B is larger than that of protein A, as depicted in Fig. 4 *B* and mathematically shown in the Supporting Material, section 2.4.2. Quite interestingly, mathematical analysis shows that when the lifetime of B is increased to become larger than the lifetime of A, another nontrivial dynamical state is possible. Namely, the two cells can be in different states at all times but with each cell oscillating in time through the different states, as shown in Fig. 4 *C*. Cells oscillate in antiphase because it is the condition for the presence of negative feedback in this circuit. Let us consider a variation with the concentration of A higher than the steady state in cell 1 and lower in cell 2. The higher A concentration in cell 1 induces more transformation of B into B^*. This results, in cell 2, in a decrease in the concentration of B, as well as in the concentration of B^* when complexation is fast enough. In turn, this tends to decrease the amount of complexed A in cell 2 and counteracts the decrease in A.

The second commonly produced network type is shown in Fig. 5. It is characterized by the fact that for a couple (A,B) of signal-sending and signal-receiving proteins, the modified signal-receiving protein B^* does not interact at

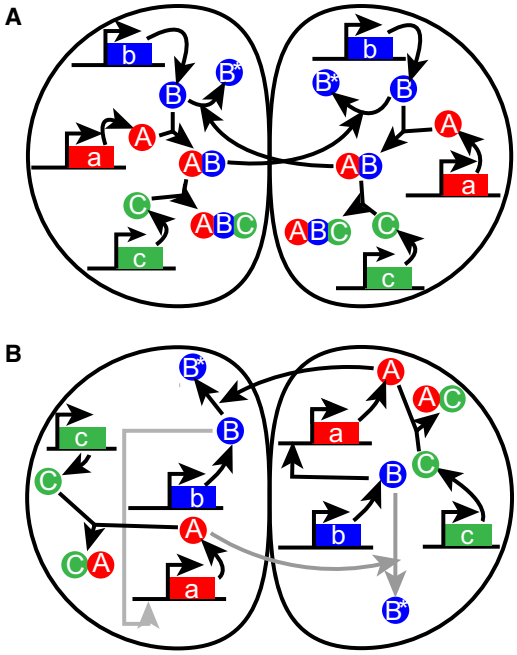

FIGURE 5 Two interacting cells with exclusive fates: type 2 networks with interaction between heterologous proteins in the signal-sending and signal-receiving cells. In this type of networks, the modified form of the signal-receiving protein does not interact with the rest of the network; the sole effect of the interaction is to lower the concentration of the unmodified signal-receiving protein. (*A*) This network, which is purely based on post-transcriptional interactions, was repeatedly produced. Note that *B* is the signal-receiving protein and that *B** has no interaction. The working principle of this network already appears quite complex but it can be understood based on our previous analysis of simpler bistable networks. Bistability is achieved when the production rate of *B* is smaller than the production rate of *A* and therefore controls the production of the complex *AB*. In this regime, transformation of *B* into *B** in a cell directly diminishes the production of the complex *AB*. Interaction between the two cells is then similar to noncooperative cross-repression between the production of the complexes *AB* in the two cells. Very similar to the AAC network, complexation of *AB* with *C* is needed to transform this monostable cross-repression, equivalent to a noncooperative self-activation, into a bistable network. (*B*) This network makes use of transcriptional and posttranscriptional interactions. The signal-receiving protein *B* stimulates the production of *A*, the signal-sending protein. Upon signal reception, *B* is transformed into *B**, which diminishes the signal-sending ability of the cell, an effect further amplified by the complexation of *A* with *C*.

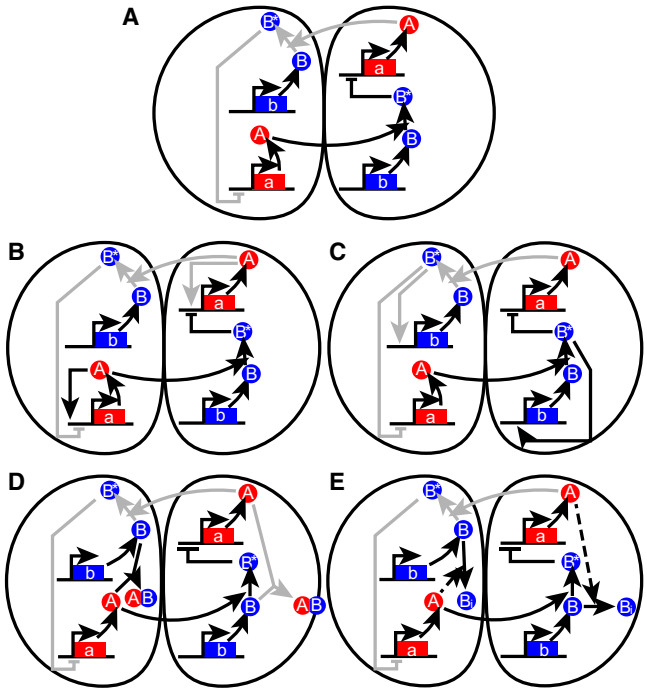

FIGURE 6 Type 3 networks: interaction between heterologous proteins in the signal-sending and signal-receiving cells relayed by an inhibitory transcriptional interaction. In this third type of networks, the modified form of the signal-receiving protein inhibits the production of the signal sending protein, at the transcriptional level. (*A*) The simplest network. Bistability requires cooperative transcriptional interactions (Hill coefficient >1). When transcriptional interactions are noncooperative, supplementary interactions are required to break the symmetry between the two cells and to render the network bistable. (*B*) Autoactivation (with a Hill coefficient of 1) of its own gene by the signal-sending protein is a possibility that was commonly observed. (*C*) Activation of the gene coding for the signal receiving-protein by the modified signal-receiving protein is also an observed case. (*D*) The addition of a complexation between the signal-sending protein *A* and the signal-receiving protein *B* is another possibility with a supplementary posttranscriptional interaction. When *A* is high in the receiving cell, this complexation prevents the existence of free *B* and signal reception (i.e., the creation of *B**). Reciprocally when *B* is high, complexation lowers the concentration of free *A* and diminishes the ability of the cell to signal. It has thus been termed mutual *cis*-inhibition in the context of the Notch-Delta pathway. (*E*) A related possibility that was also observed is that the signal-sending protein *A* catalyzes in its own cell the transformation the signal-receiving protein *B* into an inactive form B_i. In this case, there is *cis*-inhibition of *B* by *A*, but no mutual *cis*-inhibition.

all with the other genes and proteins. The interaction simply acts by depletion of *B* in the signal-receiving cell. One network of this type that was frequently produced is displayed in Fig. 5 *A*. As the network of Fig. 4 *A*, it is purely based on posttranscriptional interactions. Here, *A* needs to make a complex with *B* in the signal-sending cell to activate the transformation of *B* into B^* in the signal-receiving cell. In other words, *A* needs to be activated by *B* to be able to send a signal. Bistability is achieved when the complex *AB* can itself be further titrated into a ternary complex with a protein *C*. The network of Fig. 5 *B* makes use of a similar interaction-mediated depletion mechanism. It is based on a mix of transcriptional and posttranscriptional interactions and was also commonly produced when transcriptional interaction where restricted to be noncooperative. A variety of other more complicated networks based on the creation of a noninteracting modified signal-receiving protein were created as well in this case.

The two networks of Fig. 4 and Fig. 5 *A* are built on posttranscriptional interactions. Although less frequently (Fig. 2 *C′*), evolutionary simulations created some networks that were based on transcriptional interactions, in addition to the cell-cell posttranscriptional activation of *B*. We characterized these type 3 networks by the fact that the modified

protein B^* acts as a transcriptional regulator. B^* is a transcriptional repressor of gene a in most networks. The simplest one is shown in Fig. 6 *A* and corresponds to the textbook description of the Delta-Notch-mediated lateral inhibition. It is very similar in its principle to the simple bistable transcriptional network with cross-inhibition between two genes shown in Fig. S4.2 *B*. In Fig. 6 *A*, however, the interaction takes place between gene a in one cell and gene a in the other cell. The transcriptional interaction between the two cells is mediated by a protein B different from A in the receptor cell. That is, A in cell 1 activates B in cell 2. In turn, the activated form B^* of B represses A in cell 2. This results in A being expressed in cell 1 and repressed in cell 2 (or, of course, the reverse). As for the simple transcriptional network with cross-inhibition between two genes, bistability requires some cooperative interactions. This can be achieved, for instance if B^* represses the transcription of A in a sigmoidal manner (i.e., with a Hill coefficient greater than one).

The basic network type of Fig. 6 *A* often appeared decorated with other interactions, especially when transcriptional interactions were noncooperative. To investigate more systematically the network structures in this case, two sets of computer simulations were performed in which transcriptional interactions were restricted to be noncooperative and the mutational appearance of posttranscriptional interactions were either forbidden or possible. In the case of noncooperative purely transcriptional interactions, the two-gene network that repeatedly appeared is displayed in Fig. 6 *B*. The basic network structure of Fig. 6 *A* is supplemented by the autoactivation of A that is necessary to make the network bistable without cooperative transcriptional interactions. Here, as in several previous examples, the network is not bistable at the single cell level. In the absence of interaction, only the high A state exists. When posttranscriptional interactions were allowed in evolutionary simulations, in addition to noncooperative transcriptional interactions, they appeared added to the basic structure of Fig. 6 *A* to allow the creation of bistable networks. For instance, the network of Fig. 6 *D* displays an added complexation between A and B as compared to Fig. 6 *A*. This so-called mutual *cis*-inhibition of B by A both diminishes the availability of B in the cell where A is highly expressed and further diminishes the action of A in the cell where a expression is repressed. As a result, the network of Fig. 6 *D* is bistable even when the transcriptional repression of gene a by B has a Hill coefficient of one in contrast to that of Fig. 6 *A*, as recently pointed out and analyzed (31,32). The network of Fig. 6 *E* is analogous to the network of Fig. 6 *D* but *cis*-inhibition is not due to the formation of a complex between A and B but to a transformation of B into an inactive form B_i catalyzed by A in the same cell. In this latter case, in contrast to the network of Fig. 6 *D*, *cis*-inhibition is not mutual. Although the presence of A in a cell diminishes the concentration of B and its ability to receive a signal, the presence of B by itself does not lower the concentration of A and the cell capacity to signal.

In most of the obtained bistable networks with interaction, the symmetry between the two cells is spontaneously broken. In contrast to cell-autonomous networks, even a small initial concentration difference robustly produces two different fates in the two cells as already illustrated for the simple network of Fig. 3. Correlatively, this suggests that large differences, in the initial A concentrations between the two cells in the test phase of the evolutionary process, should ease the creation of cell-autonomous networks. The previous results were obtained with initial concentrations of A in the same range as the other proteins (see the Supporting Material, section 1.6), As shown in Fig. S4.4, more cell-autonomous networks were indeed produced when we imposed in another set of simulations, a much larger initial concentration of A in one cell and a very small concentration in the other one.

In all the previously reported simulations, interactions between cells were implemented with a simple second-order kinetics scheme. The interaction strength was thus linearly proportional to the concentration of A in the signal-sending cell and to the concentration of B in the signal receiving cell. In additional simulations, we investigated the effect of a more complex interaction scheme with a saturating dependence on the concentration on the receiving-cell side, to gauge the effect of this modification (see the Supporting Material, section 1.4). Quite generally, the types of obtained networks did not seem to be affected by this change. However, the nonlinearity in the interaction allowed in some cases the production of networks that could not have been produced without it. A notable case was the network displayed in Fig. 6 *A*, which was created in the presence of nonlinear cooperative signal transmission, even with noncooperative transcriptional interactions.

DISCUSSION

In this work, we have performed evolutionary simulations to investigate the architectures of small gene networks capable of producing different states of gene expression. We have focused on the role of interactions between cells.

Networks able to produce different cell fates from different initial conditions have been considered in a number of previous studies. Lewis et al. (6) is an early example that examines the question in the context of noninteracting cells and emphasizes the importance of multistability. The simple bistable case of a transcription factor that activates its own gene, the network of Fig. S4.2 *A*, is explicitly studied. Interactions between cells have been introduced using mathematical models (7), or along lines parallel to ours (11,17,18).

In contrast to most of these previous works, we have explicitly described posttranscriptional interactions and chosen to describe the variety of possible networks at a level

that make direct contact with biological data possible. As a result, we have obtained and analyzed several network architectures and mechanisms that have not been previously examined.

It can certainly be wondered how complete is our sample of networks and how dependent it is on the choices that, inavoidingly, had to be made in formulating the evolutionary algorithm. Although the question is difficult to fully address in the absence of a complete theory of such algorithms, the tests that we have performed lead us to believe that we have sampled most of the simplest networks. First, for cell-autonomous networks, the score function used in this work is quite different from the one previously used in (13) but the predominantly created network structures are identical. The designed score function thus does not appear to strongly bias the results. Second, we have generated and analyzed a large sample of networks to survey as completely as possible the different simple structures. Third, we have checked that varying the bounds on the number of interactions does not significantly alter the types of network with few interactions (see Fig. S4.3). Fourth, we have found that varying the difference in initial concentration between the two cells during the test phase changed the proportion of interacting versus noninteracting networks. However, the different obtained types of two-cell networks did not significantly change (compare Fig. 4 C' and Fig. S4.4 A'). It certainly remains possible that interesting network topologies have been missed. Nonetheless, we believe that we have investigated much more fully than previous works, the different possible two-cell bistable structures.

In the following, we discuss the different networks that we have obtained in light of existing biological data.

Cell-autonomous bistable networks

The main core network architectures that we have found are the familiar autoactivation of a single gene at the transcriptional level, either direct (Fig. S4.2 *A*) or indirect (Fig. S4.2 *B*), as well as the AAC (Fig. 1 *A*) and MFL (Fig. 1 *B*) networks, identified in previous studies (13,22), that make use of posttranscriptional interactions.

Biological examples of the AAC and MFL networks have previously been discussed (13,22). We simply remark, here, that complexation and sequestration in inactive complexes are known to play an important role in different cases of cell differentiation. For instance, the proneural genes *achaete* and *scute*, form various active and inactive heterodimers (33) with other bHLH proteins. The interaction between GATA-1 and PU.1 during hematopoiesis is a nice example (34) of direct protein-protein interaction, as in the AAC and MFL switches. Interestingly, the ability of protein complexation to promote ultrasensitive characteristics (35) and switch-like behavior has recently been quantitatively confirmed (36) in yeast using a synthetic gene circuit.

Bistability and different fates mediated by interaction between neighboring cells

Lateral inhibition through the Delta-Notch pathway is the most widely reported interaction (1) driving adjacent cells to different fates. Classic models include AC/VU differentiation in *C. elegans* (2) and the development of sensory bristles on the dorsal thorax of *Drosophila* (4). In a proneural cluster, one sensory mother cell emerges while its neighbors take an epidermal fate.

The central role of the Delta-Notch pathway in lateral inhibition has motivated the modeling of the classically described transinhibition (i.e., in the other cell) of *Delta* by activated Notch, both in simplified mathematical terms (7) and in a more detailed manner (8). A variety of transcriptional and posttranscriptional interactions however appear to sustend the Notch pathway, both on the cell-sending and cell-receiving sides (see (25,26) for a review). Despite much work, it is still not entirely clear which interactions are the essential ones and moreover species variation appears to exist (e.g., endocytosis of Delta appears required for signaling in flies but not in worms).

In contrast to specific modeling approaches, we have quite generally investigated the production of two distinct fates in two interacting cells. The simplest found network motif (Fig. 3) is based on a single gene a that activates its own transcription and prevents this autoactivation in the neighboring cell. A role similar to a has been proposed, in sensory mother cell determination, for the gene *scute*, which has been shown to autoactivate itself (33). Interestingly, the network of Fig. 3 is even simpler than the previously proposed two-gene model of (7). Notably, when signal saturation is taken into account autoactivation of a is not even required. It is tempting to wonder whether such a circuit existed in a primitive context.

The network motifs obtained by requiring that the two cells interact via a couple (A,B) of different proteins in the signal-sending and signal-receiving cells have been found to fall quite generally into three types depending on whether B^*, the modified protein B: i), only interacts with other proteins, ii), has no interaction whatsoever, or iii), acts as a direct transcriptional regulator.

Type 1 is realized in a network of striking simplicity (Fig. 4). It differs from the Notch pathway in that the transinhibition of A is not transcriptional but uses sequestration of A in a complex with activated B. One is led to wonder whether this mechanism will be reported in another context. Interestingly, we have found that, in a large parameter regime, this simple network produces cells oscillating in antiphase. This phenomenon has been observed in cells interacting via the Notch pathway (37) and modeled using time-delayed equations (38). The example of Fig. 4 network is an incentive to consider other possibilities.

Type 2 networks may appear quite surprising because the modified B protein has no effect in the conventional sense.

They underline a mechanism of general interest based on the depletion of the signal-receiving protein that does not seem to have been previously recognized. Being aware of it may help to recognize biological uses of this mechanism.

Type 3 networks (Fig. 6) mostly use transinhibition of gene *a* by B^*, which is the mechanism classically described as underlying Delta-Notch-mediated lateral inhibition, However, transcriptional inhibition alone is sufficient to provide bistability only when it is cooperative. Otherwise, it has to be supplemented by other interactions as found in the networks of Fig. 6, *B–E*. For instance, when interpreted with the Notch pathway terminology, the network of Fig. 6 *D* makes use of *cis*-inhibition (i.e., in the same cell) of Notch by Delta, which has been known to exist (39) for some time. Only recently however, the physiological relevance of this mechanism has been demonstrated (40). Investigations in a synthetic context (31) have further underlined its potential importance and have provided support for mutual *cis*-inhibition of Notch and Delta by titration as in the network of Fig. 6 *D*. An alternative to complexation and mutual inhibition is provided by the network of Fig. 6 *E* in which *A* catalyzes, in its own cell, the transformation of *B* into an inactive form but there is no reciprocal action of *B* on *A*. A discussion of the evidence in favor of *cis*-inhibition of Notch by Delta, of Delta by Notch, and of their possible link, can be found in (39). Recent evidence has also been found for the activation of Notch (*B*) by activated Notch (B^*) (41), as used by the network of Fig. 6 *C*.

Finally, the presented networks can usefully serve as working models of bistable multicellular networks and as a test ground for various ideas. For instance, they exhibit spontaneous symmetry breaking between the two cells that lead to amplification of small differences. A slight imbalance in initial conditions is then reflected in consistently biased outcomes as observed in *C. elegans* AC/VU differentiation in which the first born cell is found more likely to become the VU (42). As another example, a lateral inhibition network involving transcription of the Notch or Delta genes appears too slow as compared to the pattern development time in a case like fly eye patterning (43) (we thank N. Baker for emphasizing this point to us). It is perhaps reassuring and an incentive for further experiments that several of the networks presented here are purely based on posttranscriptional interactions.

SUPPORTING MATERIAL

Further discussion, methods, analyses, figures, and references are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)05403-8.

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