CoMPLEX
University College London

Mini Project 1

Design Principles of Gene Regulatory Networks in Developmental Biology

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Abstract

In the current project two extensively studied examples of pattern formation in developmental biology will be presented. Their mathematical analysis has provided evidence on the importance of GRN dynamics for robust and accurate patterning, essential for correct development. Furthermore, the main emphasis will be put on the research done for the identification of design principles for these transcription factor networks and their relevance to the real biological systems will be discussed. The two examples are the neuronal subtype differentiation in the vertebrate neural tube and the establishment of the body plan in Drosophila melanogaster from the patterning of the anterior-posterior axis. Finally, some original modelling will be presenting comparing the stripe-forming ability of mutual inhibition and cooperativity in a simple $3-gene$ network using a new theoretical framework for modelling developmental networks.

Contents

Chapter 1

Gene Regulatory Networks in Real Biological Systems

Pattern formation plays a crucial and dominant role in developmental biology. It is essential for the differentiation of equivalent cells in the early embryo development. This spatial differentiation is now known to originate from the production and interpretation of morphogens. The first major part of pattern formation during development is the morphogen, because morphogens are used by organisms to create polarity along embryonic axes [18]. In developmental biology they can take different forms and they usually are transcription factors acting on downstream genes or target genes directly. The simplest example is a temporally static decaying gradient, but there are cases where the morpogen gradient dynamics change throughout the development [1]. The second part of pattern formation, and the main discussion of this project, are the gene regulatory networks (GRNs), whose role is the interpretation of the morphogens.Gene regulatory networks are comprised of interconnected morphogen downstream genes which can be transcription factors regulating other transcription factors and target genes, forming a complex circuit. Although other mechanisms were initially believed to drive spatial differentiation in developmental biology (Affinity-Threshold model), GRNs gained ground and a lot of interest has been shown for their dynamics and architecture. It is widely accepted nowadays that these properties (dynamics & topology) are at the core of pattern formation and cell differentiation for the early embryonic development. The two main examples of pattern formation in developmental biology are the patterning of the vertebrate neural tube and of the embryonic axes in Drosophila melanogaster.

1.1 Vertebrate Neural Tube

During the early development, the vertebrate neural tube consists of neural progenitors. From the ventral midline of the neural tube Sonic Hedgehog (Shh) is secreted forming a ventral-dorsal gradient. This gradient is then interpreted to form the expression of patterns of transcription factors (TFs). The expression of these different factors establishes the discrete dorsal-ventral progenitor domains that produce the specific subtypes of motor neurons and interneurons [1]. The basic TFs controlled by the Shh gradient are the Gli TFs. Normally the Gli protein is converted to GliR, a transcriptional repressor,

but in the presence of Shh this process is blocked and GliA activator is produced. Due to the fact that there is a Shh gradient present in the neural tube, high near the ventral region and low near the dorsal, two opposite gradients of GliA and GliR are formed [4].

The GRN of downstream Shh target genes is considered to play an essential role in this patterning. To see why the logic of the cross-regulatory network is indeed important we must first consider a classical model of morphogen pattern formation called the Affinity-Threshold model (AT) [22]. In this model there is positive correlation between the expression range of morphogen target gene and their binding affinity. In the current systems this would mean that genes expressed more ventrally, where the concentration of the morphogen is high, must have lower affinity whereas genes that are expressed more dorsally must have higher binding affinities in order to be able to be expressed in regions where the morphogen concentration is low. Consequently, increasing the binding affinity would increase the range of expression of all genes and lowering the concentration would decrease it.

For the neural tube such correlation between expression range and binding affinity was not found [4]. Experimental data using in vivo reporter of Gli activity showed that although more ventrally expressed genes reacted to binding affinity changes according to the Affinity-Threshold model, the more dorsally expressed genes did not. Decreasing the binding affinity increased the expression of these genes. These data showed a clear discordance to the AT model. This could be due to the fact that there are two opposite gradients acting at the same time and could be partially be explained by differences in the cooperativity or binding affinity of Gli isoforms. However, there is no evidence of different cooperativity or affinity of Gli isoforms [4] and also the observation of the reporter activity over the course of development showed that the activity of some genes cannot be explained by fixed threshold of Gli activity [1]. These can be an indication that a different mechanism is needed to explain the observed spatial differentiation.

In addition, mathematical and computational modeling provided in silico evidence that the architecture of the downstream network was not only able to interpret the morphogen correctly but also to give robustness and accuracy to the patterning. The mathematical model used was a known statistical thermodynamics formulation [24]. In this formulation the probability of a gene being turned ON is given by the ratio of all ON states over all possible states, where ON signifies the gene being expressed. The states are the bound configuration of the DNA to the RNA and to the TFs [3].Then, the concentration of the gene products is found using an ODE that includes the effect of production and degredation.

$$P_{bound} = \frac{\sum_{i=stateswithPbound} \omega_i}{1 + \sum_{i=allboundstates} \omega_i} \tag{1.1}$$

$$\frac{d[C]}{dt} = \alpha[C] - \beta[C] \tag{1.2}$$

Where $\omega_i = \prod_\mu K_\mu[X_\mu] \prod_{\mu,\nu} c_{\mu,\nu}$. K_μs are the binding affinities, $c_{\mu,\nu}$ is the cooperativity between two occupied sites, μ, ν are the states bound in state i, α is the production rate of proteins, β is the degradation rate and finally [C] is the protein concentration of each transcription factor [3].

Using this model, it was possible to simulate the combinatorial effect of two opposite transcriptional effectors (GliA, GliR) and of the specified GRN. Two independent binding sites were used for the effectors, to provide non-linearity. The GRN was comprised of four mutually repressive genes (Olig2, Nkx2.2, Pax 6, and Irx3). Then using Bayesian methodology the stripe-forming parameter space was found by comparison to the wild-type gene expression patterns [3].

The model was able to both reproduce the experimentally observed behavior occurring when the binding affinity changes and the sharp boundaries of gene expression. Furthermore, the system displayed hysteresis, meaning that after a steady state has been reached it can be maintained even if the signal is reduced[3]. Hysteresis is memory and confers robustness in case of decrease of the signaling gradient. In addition to the above, another modeling approach was used for the description of the neural tube GRN providing again evidence that the accurate and robust patterning is an emergent property of the underlying network [1]. This other model, despite the fact that it used a different mathematical approach, again shows hysteresis and proves that the network acts as a buffer to signal fluctuations, both essential for in vivo reliable patterning. So, it is clear from both the experimental data and the modelling analysis that GRNs are crucial for the development of the neural tube.

1.2 Drosophila Melanogaster Body Plan

One of the most widely studied examples of developmental patterning is the anterior-posterior (head-tail) axis patterning in Drosophila melanogaster. This patterning determines the different body parts of the adult Drosophila. There are three main gene types involved in this procedure, maternal effect genes, segmentation genes and homeotic genes. The maternal effect genes form the protein gradients that are essential for the patterning. These genes include Bicoid (Bcd), Caudal and Nanos. The first two are mainly related to anterior development whereas the last is related to posterior. Bicoid and Nanos act as morphogens of opposite polarity, anterior posterior and posterior-anterior respectively [19]. The second type of genes, segmentation genes, are themselves divided into three categories, gap, pair-rule and segment-polarity genes, all responsible for the final segmentation of the embryo. Gap genes are first transcriptionally regulated by maternal effect genes to establish the primary body plan dividing the embryo in large parts. Gap-genes act as transcription factors for pair-rule genes which divide the embryo in pairs of segments. Finally pair-rule genes regulate segment-polarity genes which form the final body segmentations. These different segments assume their identities through the final type of genes, homeotic genes, whose role is the transcriptional regulation of genes responsible for anatomical structures [5].

Manu et al. explored the ability of Drosophila embryos to reduce the phenotypic variations [27]. Their analysis revealed that in the core of this process lies the cross regulation of gap genes and that the Bicoid gradient alone was unable to produce the gap gene expression borders with the observed variance. A gene circuit was built with differential equations describing the protein production, degradation and diffusion of the gap genes hb, Kr, gl and kni. The model circuit shows scaling with respect to egg length and predicts the variance of the gap gene expression borders. Experimental data from double mutants for Kni and Kr showed that the deviation of the location of the anterior hb border and the posterior gl border increased markedly. They also found that the borders are usually set by one activator and one or two repressors and that the gap gene network is created by mutual repression interactions among the genes comprising it. To sum up, the modeling revealed that the small variance of the borders, vital for the correct development of the embryo, was a property of the GRN. The experimental data were in agreement to these results, further supporting the essential role GRNs play in developmental patterning.

Chapter 2

Design Principles

The importance of GRNs has led many people to start searching in order to identify design principles of these networks. To support this approach there are papers suggesting that the dynamics of larger networks can be simulated using only a few essential components and that larger network are created by the combination and repetition of smaller networks [12][16]. Design principles are common modular features repeated in many networks vital for, improving stripe-forming ability, robustness and evolvability, and they can be specific network motifs (e.g. Feed Forward Loops) or gene interactions (e.g. auto-regulation ,feedback, mutual repression). Computational modeling of ODEs describing GRNs play a major role in the research for design principles but it is very important to find a connection between the in silico results and the in vivo observations.

In a well known paper by Sharp and Cotterell [6] an atlas of gene regulatory networks was built using computational modeling. To search the design space a mathematical model describing the gap gene patterning was used whose parameters include strength of interaction between genes, sign of interactions (inhibition or activation), degradation rates and finally cell-cell communication. Furthermore, molecular noise was added to the model to make it more realistic, as robustness to noise is essential for real biological system. The main purpose of the paper was to identify single stripe-forming networks with no specific stripe width or location. To this end, all the unique 3-gene topologies were created and simulated using 30000 randomly chosen parameters for every topology. From a total of 9710 topologies only 471 produced the desired phenotype. For these 471 different topologies a complexity atlas was created and six distinct stripe-forming mechanisms were found, three of which are used in real biological systems as shown in the figure 2.1 (Cotterell et al. 2010[6]).

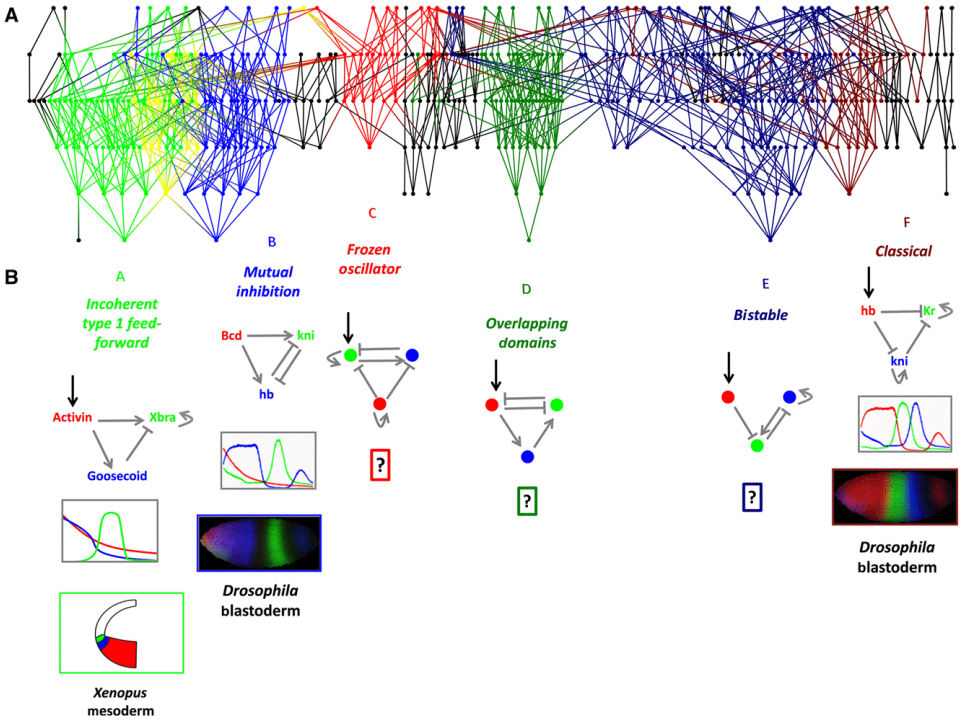


Figure 2.1: Six mechanisms - Cotterell et al. 2010)

Only one of the six mechanisms made use of the cell-cell communication, where the loss of communication between cells rendered the systems useless. The three main design features employed by the six mechanisms are feed-forward, auto-positive feedback and negative feedback. A very interesting observation is that four out of six mechanisms rely on feed forward topology which has been identified as an important feature in other papers as well, as we shall see later. In that specific feature the 3-gene network has the ability to split the input into distinct channels and can be reduced to a 2-gene network as the first gene, the one that reads the morphogen, mimics the behavior of the morphogen gradient. Hence, the same mechanism can be produced by using two genes and multiple morphogen inputs. What this shows is that the overabundance of the FFL in this design space exploration could point to a different design principle, not directly related to GRNs, which is the use of multiple morphogen inputs [6]. Furthermore, another feature identified in this paper, which is used in many real biological systems, is the mutual inhibition topology. This is observed in both the Drosophila and the Neural tube patterning discussed previously. The main benefits identified by this paper are the ability to reach equilibrium very fast, as well as the evolvability of the motif, since this can be used multiple times in larger networks to give rise to many behaviors. Mutual repression and more generally negative feedback, which is used by the majority of the mechanisms identified here, is again one of the features found in a number of papers about design principles and seems to be essential for developmental patterning. Finally, positive auto-regulation plays a major role in the majority of the six mechanisms by providing bi-stability and additional robustness to the gene expression.

The three main design principles identified by Sharp & Cotterell, FFL, mutual repression and auto-regulation, deserve special attention as several papers have praised their

role in pattern formation. It is important to point out that the difference between the three designs is that FFL is a specific topological motif, whereas auto-regulation and mutual repression are gene interactions within the network. As we will see, these interactions can be added to FFLs to enhance their stripe-forming ability, as was the case of the above-mentioned paper. The mutual repression mechanism was essentially a FFL with an added negative feedback between the two downstream genes.

There are eight distinct feed forward loops, four coherent and four incoherent, categorized according to the sign of the interactions between the three genes that comprise them. There is one target gene and two pathways. If the pathways have opposite effects on the target gene then the feed forward loop is called incoherent, otherwise it is called coherent (Figure 2.2 Ghosh et al. 2005 [10]). FFLs have been considered to be special since statistics cannot account for their over-abundance in natural systems. Widder et al. reconstructed the natural abundance of FFLs in transcriptional networks with their analysis [28]. They showed that the plasticity of these motifs accounts for this phenomenon, where plasticity is defined as a compromise between specialization and flexibility and is directly related to evolvability which is defined as a tradeoff between robustness to mutations and the capability to modify their function due to mutational pressure [28]. These features imply that FFLs could be considered design principles for TF networks in general as well as for developmental systems in particular.

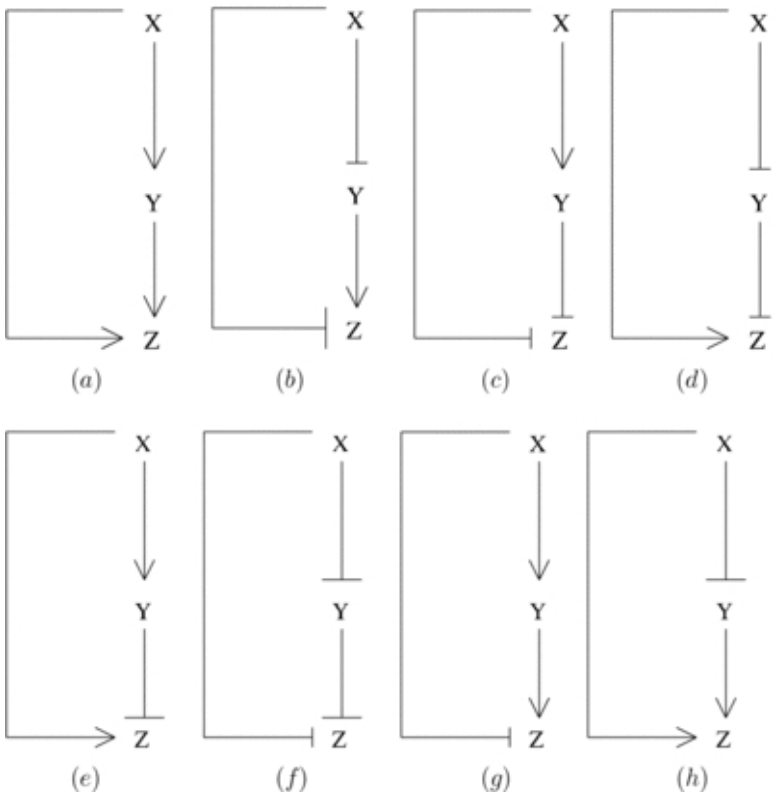


Figure 2.2: Eight Feed Forward Loop Motifs - Ghosh et al. 2005)

IFFLs have been identified as single stripe generators in several developmental systems [11]. The combination of IFFL and a feedback, capable of inducing morphogen

response from the source, provides a stabilization of the expression peak in situations of increasing or decreasing morphogen input, with application in real systems such as the vertebrate limb bud development [11]. Finally, it is worth mentioning that there are some recent papers that have identified IFFLs as the minimal network motifs for stripe formation [23][20][17]. In one of the most recent papers, the four distinct motifs were also built synthetically and tested, in order to show that they correspond to the minimal motifs capable of producing four unique mechanisms found in more complex 3-node networks [23]. Despite the common reference to IFFLs in these papers, we should notice that there are some observed differences which might be due to the different mathematical models used to describe the expression of the genes.

Inhibitory interactions, and more specifically mutual repression seem to be a very dominant design principle, observed in many real biological systems and tested via mathematical and computational modeling.These interactions seem to be capable of providing spatially restricted gene expression [8]. Sokolowski et al. simulated the effect of mutual repression using an example from the anterior-posterior patterning of Drosophila [26]. In their model they performed stochastic simulations of a minimal model of repression between two gap genes, hb and kni. Hb and kni were activated by the maternal regulators Bicoid and Caudal respectively. They compared the results with a model using only one gap gene activated by a single morphogen. Their findings showed that mutual repression enhances robustness against intra-embryonic fluctuations and embryo-embryo variations in morphogen levels. This is in accordance to experimental observations of double mutant embryos lacking the mutual repression between those genes [26]. To further support the usefulness of mutual repression they varied several parameters (mutual repression strength, diffusion constant, hill coefficient, maximum repression level) and observed the different effect each parameter played in steepness and robustness. Remarkably mutual repression was able to increase the steepness without increasing the noise of protein expression, whereas there was a compromise between steepness and noise (precision) when the cooperativity was increased (increasing hill coefficient) or when the diffusion constant was lowered. The positive effect of adding mutual repression to network motifs was also explored by Ishihara & Shibata [13]. Initially, a database search indicated that mutual inhibition interaction is very common during the early segmentation of the Drosophila embryo. Then by mathematically modeling the effect of mutual interactions they concluded that mutual repression produces sharp and high expression levels without affecting the location of the stripe.

A more general exploration of the role of feedback was done by Munteanu et al. [17]. They tested the effect of adding feedback in incoherent feed forward loop motifs finding some very interesting results that further support the crucial role of mutual inhibition in particular. An atlas was created by adding all possible feedback to the two downstream genes of the I1-FFL and I3-FFL (The first and third motifs in figure 2.2 respectively). No feedback was added to the morphogen reading gene so as not to alter the feed-forward mechanism. A parameter sampling was then conducted in order to determine the stripe-producing parameter space. The results showed that with the addition of inhibition between B and C, thus creating mutual inhibition, there was a very high parametric volume boost with increasing strength of mutual repression. A maximum value was then

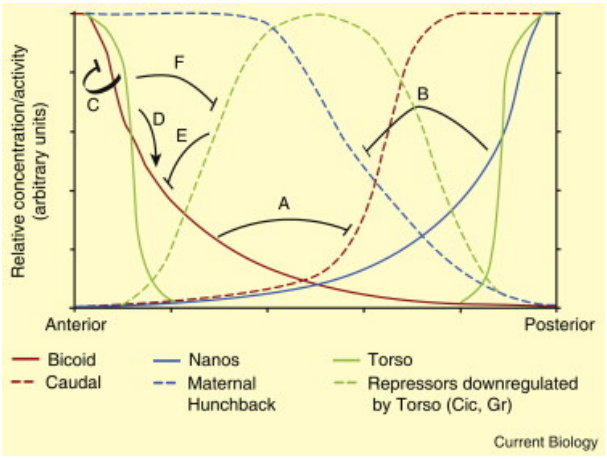
reached, and increasing the BC interaction further decreased the parametric volume, but this decrease was due to parameter space limitations rather than a property of the mutual repression motif. Although adding positive feedback to I3-FFl had no effect on the parametric space, mutual inhibition in I1 increased it offering robustness and allowed the system to take new parameter values that produce sharp stripe borders [17].

Regarding auto-regulation, J.P. Lopes et al. provided experimental and mathematical evidence of its ability to confer switch-like behavior and hence sharpness [15]. They used as a base the anterior-posterior axes development of Drosophila, and specifically the expression of the hb gap gene. From experiments they determined that embryos that are homozygous for a particular allele, responsible for producing proteins incapable of binding to DNA (no auto-regulation), showed an obvious decrease in sharpness and a shift in the position of the border, in contrast to wild type or heterozygous embryos. By developing a reaction-diffusion mathematical model of hb expression, including self-regulation and cooperative binding of bcd to hb, they were able to produce sharp borders of hb expression. In case of loss of the self-regulation reaction, bistability was also lost along with sharpness. Furthermore, although bcd activates and controls the position of hb expression through cooperative binding it is not sufficient to produce sharp borders.

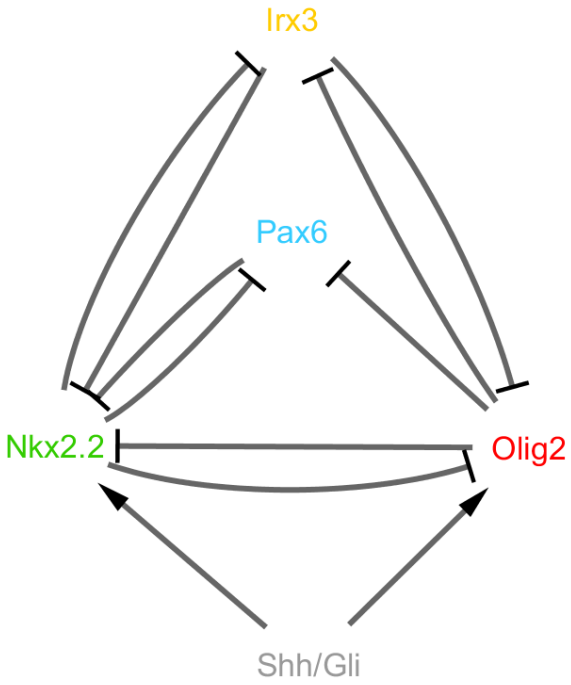
In a more general view regarding design principles, P. Francois & D. Siggia used in silico evolution and mutual entropy to predict embryonic patterning and were able to observe some general design principles of GRNs [9]. To this end, they used mutual entropy as a fitness function, which is a function of the concentration of genes that define cellular identity (realizators) and then they ran a mutation-selection evolution algorithm. Properties of the fitness function are that it favors diversity, i.e. number of realizators, and uniqueness, i.e. a single cell must express only one realizator. They examined two cases. In the first case the genes were exposed to a static morphogen gradient that vanished just before the end of embryogenesis and in the second to a sliding morphogen gradient in order to model pattern formation during growth. In both cases they observed that realizators were auto-regulated so as to keep their expression high after the disappearance of the gradient. This was achieved by means of bi-stability due to auto-activation, as mentioned before. Moreover, the posterior boundaries of genes were controlled by repression from other genes posterior to them. These findings are in accordance with other papers focusing on the importance of cross-repressive interactions and auto-regulation.

This chapter about design principles would be incomplete without a discussion of these principles in the context of real biological systems discussed. Mutual inhibition and more generally cross-repression (negative feedback) among network genes seems to be a very common interaction. Apart from gap genes, cross-repressive interactions of larger networks are also found in the vertebrate neural tube patterning [4]. The theoretical models described previously regarding the neural tube development used a network of four genes all connected to each other via negative inhibitory interactions and were able to produce the desired behavior, as mentioned already (figure 2.3 B Cohen et al. 2013). The cross-repressive system of the four genes can also be viewed as a system containing 4 IFFLS, two I1-FFLs that link Gli to Nkx2.2 and to Olig2 and two I2-FFLs that link Pax6, Nkx2.2 and Olig2 [1]. Furthermore, the relevance of these principles to real systems

is evident from the fact that many of the theoretical papers used real examples for their modelling and included experimental explorations confirming their hypothesis, such as the auto-regulation of hunchback in Drosophila, the mutant Drosophila embryos lacking the mutual repression interaction between kni and hb as well as the database search regarding the mutual interactions of gap genes during segmentation of the embryo.



(a)



(b)

Figure 2.3: (a) Expressions and interactions among genes in drosophila patterning - Porcher & Dostatni 2010. (b) Neural tube ragulatory network

The approach that GRNs seem to drive the pattern formation during embryonic development and is gaining more and more ground. Experimental work gives the opportunity to observe the behavior of wild type and mutant embryos supporting the role of GRN dynamics and architecture in interpreting the positional information of morphogens. Moreover, mathematical and computational modeling provides insight to the dynamics and properties of these networks, unavailable using any other method. Also, with the cooperation of life and natural sciences it has been made possible not only to explore the design space of GRNs but also to built and test them synthetically. A short exploration of some design principles mentioned follows using a similar model to the one used for modeling the neural tube.

Chapter 3

Modelling of A Three-Gene Network-Mutual Repression vs Cooperativity

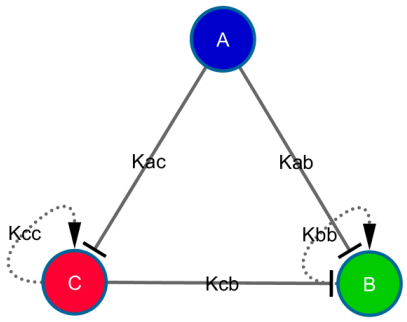


Figure 3.1: Transcriptional factors network

A $3-gene$ network, depicted above, was used to explore the different effects of cooperativity and mutual inhibition.A is the morphogen reading gene and B is the target gene.The mathematical framework used to describe the concentration of proteins is different from the classical Michaelis-Menten formulation and is a simplification of the thermodynamics ensemble model described in the neural tube patterning section. A similar exploration was done in a paper by [26], using another mathematical formulation. Their results showed that, although both mutual repression and cooperativity were able to steepen the borders of gene expression, only mutual repression displayed precision by not increasing the noise in the expression of genes. In the model presented here mutual repression is not able to produce sharp borders and stripe-like behavior in contrast to cooperativity which seems to be a promising design principle.

3.0.1 Methods

For the description of the protein concentration of the $3-gene$ three ODEs are used representing the evolution of the concentration with respect to time. In the rhs there

are two terms. The first term is the production of the protein and is given by the probability of the gene being expressed, i.e. the number of ON states over the total number of states, and the second term the degradation of the protein. Moreover, the concentration has spatial dependence due to the dependence of the morphogen reading gene, A, to the morphogen gradient. The gradient is static and decaying from the source (zero). Regarding the simplification, no mRNA dynamics were used and the degradation rates are the same for the three genes. The main reason for these simplifications is the belief that the stripe-forming ability of these systems can be represented by just including the protein dynamics and that the dominant parameters in stripe-formation are the interaction rates between the genes. Furthermore, including the full dynamics and the different rates would overcomplicate the simulation and the interpretation of the results. The evolution of the protein concentration is given by the following equations:

$$\frac{\partial[A]}{\partial t} = P_A - d[A] \qquad P_A = R_A \frac{k_M[M]}{1 + k_M[M]},$$

$$\frac{\partial[C]}{\partial t} = P_C - d[C] \qquad P_C = R_C \frac{k_{CC}[C]}{1 + k_{CC}[C] + k_{AC}[A]},$$

$$\frac{\partial[B]}{\partial t} = P_B - d[B] \qquad P_B(x) = R_B \frac{k_{BB}[B]}{1 + k_{AB}[A] + k_{CB}[B] + k_{BB}[B]}.$$

Where $[M]$, the concentration of the morphogen, is constant in time and decaying exponentially in space.

$$[M] = M_0 e^{-\lambda x}.$$

To explore the two cases, additions were made to the above equations. For the mutual repression a term was added in the denominator of P_C representing the feedback of B, $K_{bc}[B]$. For the cooperativity the concentration [A] was raised to the power of n,m on the denominator of P_C and P_B respectively and [C] was raised to the power of l in the denominator of P_B. The values of n,m were 6 and 2 respectively and the value of l was 5.

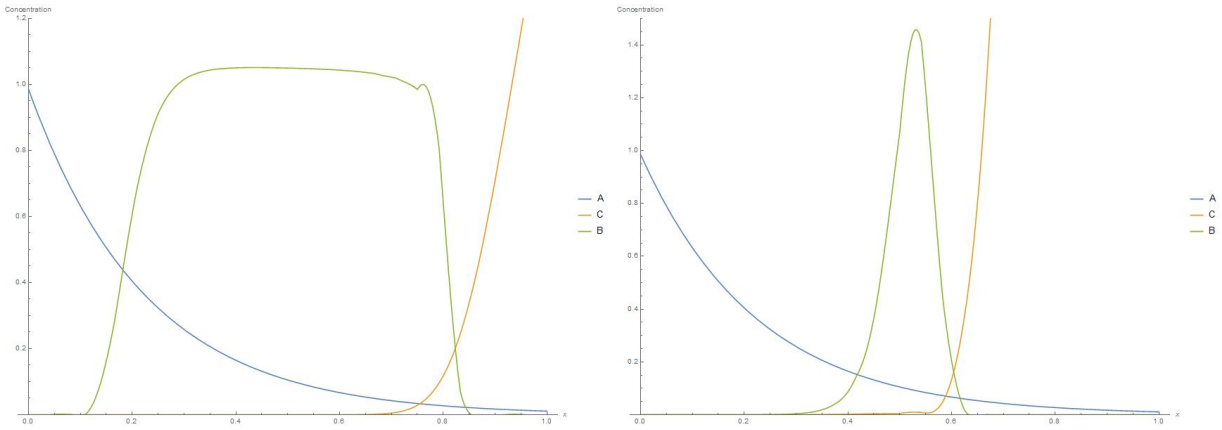
The stripe-forming ability was tested by means of Monte Carlo simulations. Initially a scoring function was created to score the stripes. The desired phenotype for a stripe is a Low region followed by a High region in the middle and then again by a Low region. The scoring function works as follows. It computes the value of $B[x,t]^2$ for the low region. When the concentration goes above 10% of the maximum value of B[x,t] the first Low region stops and the High region begins. In that region the scoring function calculates $(B[x,t]-1)^2$ and when that value falls below 10% the second Low region starts and again $B[x,t]^2$ is calculated. The final score is given by the addition of the scores in these three regions.The purpose of variable boundaries in the scoring function accounts for stripes of different width. To summarize:

$$LowRegion : s1 = ([B])^2, 0 < x < x_1 where, B[x_1] > 10\% max\{[B], 0 < x < 1\}$$
$$HighRegion : s2 = ([B] - 1)^2, x_1 < x < x_2 where, B[x_2] < 10\% max\{[B], 0 < x < 1\}$$
$$LowRegion : s3 = ([B])^2, x_2 < x < 1.0$$
$$Score = s1 + s2 + s3$$

The final step was the Monte Carlo simulation. To this end, 30000 different sets of parameter values were sampled using a logarithmic distribution, since a change in small values of parameters has greater effect than changes in larger values [6]. As it turns out, from a preliminary investigation, the parameters controlling the auto-regulation of B and C have a very narrow range. Outside of this range the concentration is very small or very large. For the rest of the parameters, the range was three order of magnitude for both cases. Finally, the two features were run separately producing 30000 pairs of scores and parameters each.

3.0.2 Results

The results of the simulation indicated that cooperativity can be a very strong design principle of GRNs and arguably better than mutual repression regarding sharp boundaries. To support this, the first analysis of the results showed that cooperativity achieved a much better (lower) score than mutual repression, 4 versus 13. Moreover, mutual repression produced pulse-like stripes which were not the desired outcome whereas cooperativity displayed stripes with sharp boundaries.



(a) (b)

Figure 3.2: (a) Protein concentration of 3-gene network with cooperativity. (b) Protein concentration of 3-gene network with mutual repression between B and C.

Further analysis established even more the advantage of cooperativity over mutual inhibition. Setting a score equal to 20 as an upper bound for the stripe-forming region of the parameter space, it was found that 230 different parameters sets were able to produce a lower score with cooperativity in contrast to 23 with mutual repression. This could be an indication that cooperativity is much more robust. Apart from the comparison

some analysis was made to explore the correlation between the score and the different parameters in both cases. The resulting graphs are shown in figure 3.3, where the x axis is in logarithmic scale.

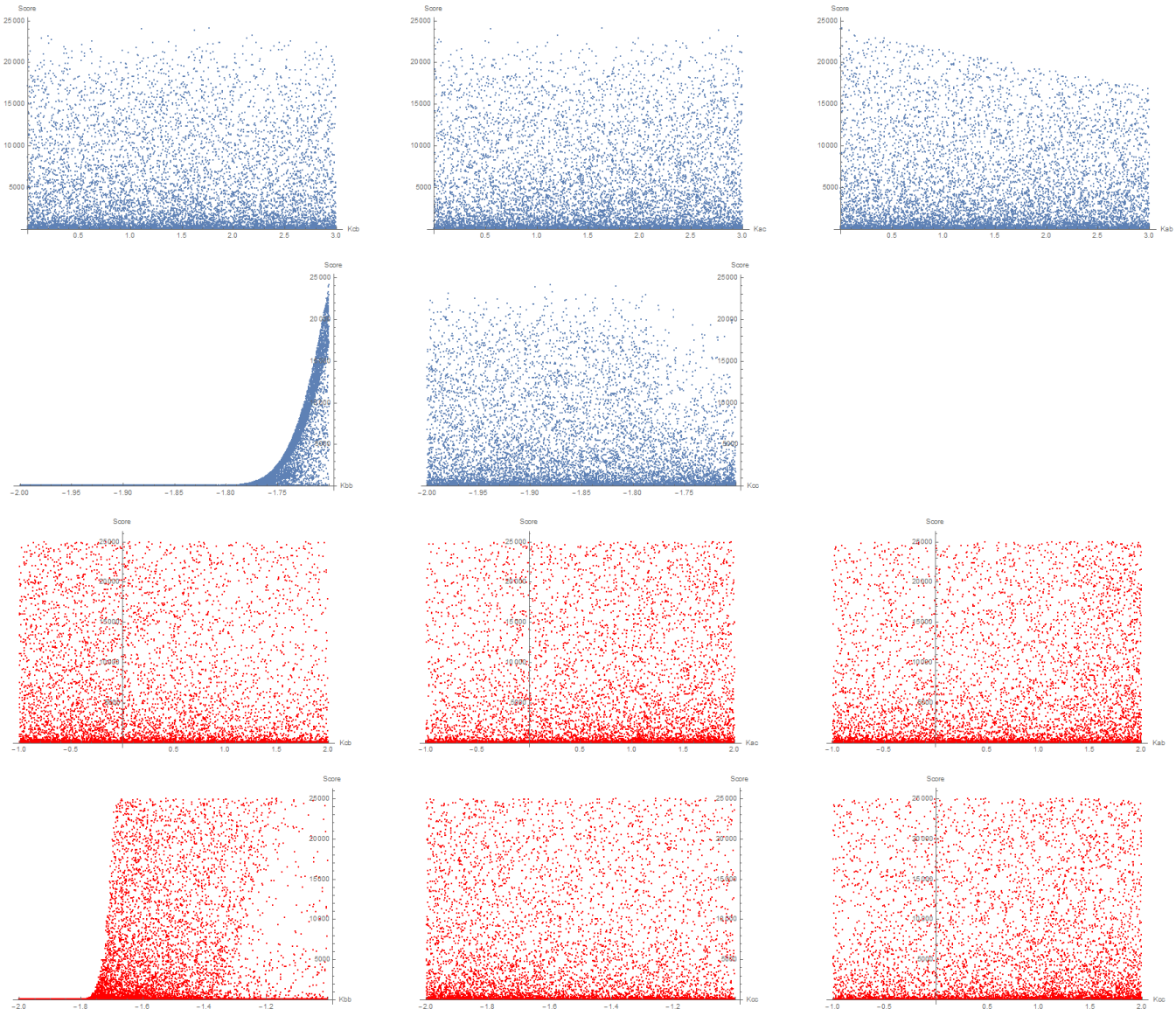


Figure 3.3: Correlation of scores and parameter value range in logarithmic scale.

No correlation is observed in most of the parameters for both cases except K_{ab} in cooperativity and K_{bb} in both cooperativity and mutual repression. There seems to be a decrease of the upper limit for the scores, hence an overall improvement, as the strength of the interaction between A and B is increased but it is a weak relationship. The strong correlation observed with auto-activation parameter is a consequence of the scoring system and of the use of the simplified model, rather than an intrinsic property of the topology, as will be discussed later.

Finally, with the use of histograms of the stripe-forming parameter sets (score below 20) for the cooperativity case, the distribution of values was found, displaying a corre-

lation between low score and values in the lower end of the available range. That was evident in all three interaction rate parameters. This analysis was not conducted for mutual repression due to the limited amount of data in the stripe-forming region.

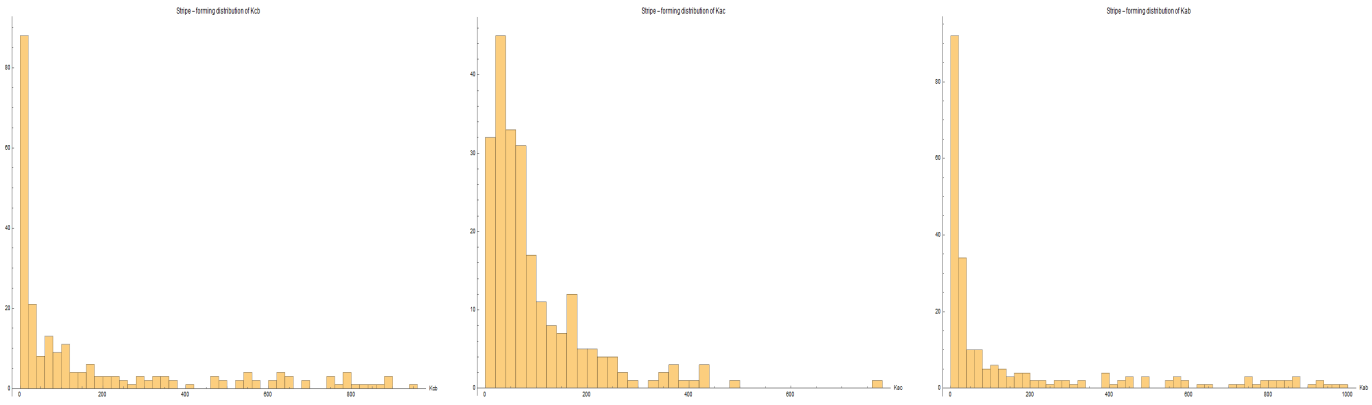


Figure 3.4: Histograms of parameter values in the stripe-forming region, where the score is below 20.

3.0.3 Discussion

The results show that in this new mathematical framework cooperativity could be an important feature of GRNs. Although the results did not support the importance of mutual repression discussed in the review it must be pointed out that cross-repressive interactions are essential for the correct expression of multiple gene networks, like the neural tube patterning, and that the topology of the network can also play a vital role. Another aspect of the modeling that needs attention is the scoring system, for example there where pulse-like stripes in the mutual repression case that had better scores than normal stripes in the cooperativity case. Obviously this is not desirable and it is an indication of the deficiencies of the scoring function. Furthermore, although it was possible to correctly score stripes with different widths but this was not the case for different heights. That posed a problem to the range of K_{BB} and K_{CC} since they had to be restricted in order to produce stripes with height close to one. This is also a problem created by the use of the simplified ODEs. Using the full model, which includes mRNA dynamics, poses a natural restriction on the height of protein expression without the need for very narrow auto-regulation parameter ranges. Finally a more efficient simulation method is needed. With the Monte Carlo simulation a large parameter space is explored to find a much smaller functioning parameter space. Different methods that can be used and can be more effective, in this context, are the Sequential Monte Carlo and the Approximate Bayesian Computation.

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