5

Mathematical Modelling of Gene Regulatory Networks

Ana Tušek and Želimir Kurtanjek
Faculty of Food Technology and Biotechnology, University of Zagreb
Croatia

1. Introduction

Living cells can be observed as complex dynamical systems that are constantly remodelling themselves as response to changes in their environment (Zak et al., 2005). The cell metabolism includes number of reactions and products of reactions which interact forming a metabolic network. The aim of modern biology is to understand the structure and dynamic of those complex interactions.

Due to the fact that large amount of date about processes in living cells are being collected every day, it become necessary to use computers for data processing and analysis. Introducing computer technologies into biology new discipline has been develop, systems biology or computational biology. The aim of system biology is describing and understanding how parts of organism interact in one complex system; systems biology aims to develop mathematical model of biological systems by integrating experimental and theoretical techniques (Hecker et al., 2009, Albert, 2004). Systems biology studies biological systems by systematically perturbating them (biologically, genetically or chemically) monitoring the genes, proteins and informational pathway response (Strizh et al., 2007). According to Bruggeman & Westerholl, 2007 a complete systems biology approach requires (i) characterisation of organism molecular composition, (ii) components dynamics (spatial and temporal) and (iii) detail analysis of molecular response to internal and external stimuli.

Progress in molecular biology led to development of complete maps of genomes of many organisms; it is also possible to identify and classify proteins. Although the number of completely sequenced genomes is mounting rapidly, our knowledge of transcription regulation is limited to a few model organisms (Janga & Collado-Vides, 2007). The interactive regulation of genes, working together to create gene networks has been considered the origin of many functions of organism (Mochizuki, 2008). Classical molecular method (Northern blotting, reporter genes and DNA footprinting) have provided great insight into regulatory relationship between genes; advancement in genetic experimental technologies DNA microarray analysis provide an effective and efficient way to measure the gene expression levels of up to tens of thousands of genes simultaneously under many different conditions (Xu et al., 2007). The control of the gene transcription is an integrated mechanism involving the interaction of genes and proteins (Knott et al., 2010). Every gene

has one or more activators and one or more inhibitors that are regulating the specific gene expression, depending on the situation in the cell and cell environment. The complex network of genes and there activators and/or inhibitors is defined as gene regulatory network. Gene regulatory networks can be very usefully for understanding the organisation within cells, because in gene regulatory networks information form the cell state and the outside environment are translated into correctly timed gene expression (Crombach & Hogeweg, 2008). Gene regulatory networks are usually described as network models where the dependencies between genes are presented by direct graph, in which nodes represent genes, proteins, enzymes or other chemical substances and edges led form a regulator to its target (edges represent transformation, eg. phosphorylation and dephosphorylation, or activation and deactivation)(Wilczynski & Furlog, 2010; Dilão & Muraro, 2010). Ideally, gene regulatory network display flow of information throughout embryogenesis (Hunman et al., 2009). To easily analyse those complex systems, mathematical models of gene regulatory networks have been developed. Mathematical models of gene regulatory networks include set of differential equations, graphical networks, stochastic functions and simulation models. Models can be used for making novel predictions and to plan future experiments.

In this chapter the theory of gene regulatory networks will be presented. The chapter will start with ideas how gene regulatory networks are constructed. There will be data on different types of gene regulatory networks and approaches for modeling those systems. This chapter will try to explain why is the modeling of complex regulatory networks important for genetic engineering and how can the mathematical analysis of gene regulatory networks be used for genetic engineering experiments planning and results interpretation.

2. Genes and genome

The hereditary nature of every living organism is defined by genome. Genome is formed of long sequences of DNA that provide information necessary to construct organism (Lewin, 2004). So genome can be divided into series of DNA sequences called genes. Each gene represents single protein (there is relationship between the base sequences of a gene and the amino acid sequence of the polypeptide whose synthesis directs the gene) (Berg, 2001). Genome of living organism can contain from less than 500 genes (for mycoplasma) to more than 40 000 genes (for human genome) (Table 1).

Phylum	Species	Genome (bp)
Algae	*Pyrenomas salina*	$6.6 \cdot 10^5$
Mycoplasma	*M. pneumoniae*	$1.0 \cdot 10^6$
Bacterium	*E. coli*	$4.2 \cdot 10^6$
Yeast	*S. cerevisiae*	$1.3 \cdot 10^7$
Slime mold	*D. discoideum*	$5.4 \cdot 10^7$
Nematode	*C. elegans*	$8.0 \cdot 10^7$
Insect	*D. melanogaster*	$1.4 \cdot 10^8$
Bird	*G. domesticus*	$1.2 \cdot 10^9$
Amphibian	*X. laevis*	$3.1 \cdot 10^9$
Mammal	*H. sapiens*	$3.3 \cdot 10^9$

Table 1. The genome size of some organisms (Lewin, 2004)

The number of genes in genome can be identified in several ways: (i) by defining open reading frames, (ii) by identifying all the mRNAs (transcriptome) or (iii) by identifying all the proteins (proteome). Due to the fact that some of the genes are presented in more then one copy or are related to one another, the number of different types of genes is less than total number of genes.

Over the past decade genome sequencing has generated large amount of new information. The main goal in sequencing is the identification of molecular and cellular function of all gene products. Interpretation of raw DNA sequences data includes identification and annotation of genes, proteins and metabolic and regulatory pathways (Médigue & Moszer, 2007). Accurate annotation of the human and genome of other organisms is essential for drug discovery (Rust et al., 2002). The mostly used annotation method is sequence homology recognition. According to Yakunin et al., 2004 apart sequence-based method, few others approaches can be used: (i) analysis of temporal, spatial and physiological proteins regulation, (ii) analysis of protein interactions, (iii) analysis of gene neighborhood, (iv) analysis of gene knockout phenotype, (v) analysis of the protein activities, (vi) analysis of post-translational modifications and (vii) protein structural analysis.

More information about components interaction allows multidimensional annotation; one dimensional annotation includes identification of genes in genome and description of functionality; two dimensional annotation specifies the cellular components and their interactions; three dimensional annotation of genome includes description of intracellular arrangement of chromosome and other cellular components, while four dimensional genome annotation could include changes in genome sequences due to the evolution (Reed et al., 2006). Genome annotation is usually preformed using one of the bioinformatics tools, GLIMMER, GlimmerM and GENSCAN (those programs include gene finding algorithm) or BLAST, FASTA and HAMER (sequence-homology search tools) (Reed et al., 2006).

2.1 Gene regulation

Taking in account central dogma of molecular biology developed by Francis Crick (transfer of sequence information between different biopolymers: RNA, DNA and proteins) there are three possible places of regulation of production of an active gene; first is the transcriptional regulation, second the translation regulation and the third post-translation or post-transcriptional regulation (Fig.1.). Regulation of gene expression is fundamental for the coordinate synthesis, assembly and localization of the macromolecular structures of cells (Halbeisen et al., 2007).

Regulation of gene expression at transcriptional level is evolutionary conserved mechanism in all cellular organisms. This process is mediated by physical interactions between transcription factors and *cis*-acting regulatory elements in promoter region of target genes (Janky et al., 2009). During transcription, mRNA is synthesized using mRNA polymerase. This process can be divided in four steps: (i) promoter recognition, (ii) chain initiation, (iii) mRNA chain elongation and (iii) chain termination and regulation can occur at each step.

Protein synthesis occurs during the translation process; mRNA is "translated" into specific polypeptide according to rules of tri nucleotide genetic code. This step of protein biosynthesis can also be divided into three parts: (i) initiation, (ii) elongation and (iii) termination. Each of these phases requires a specific group of translocation factors (Day & Tuite, 1998).

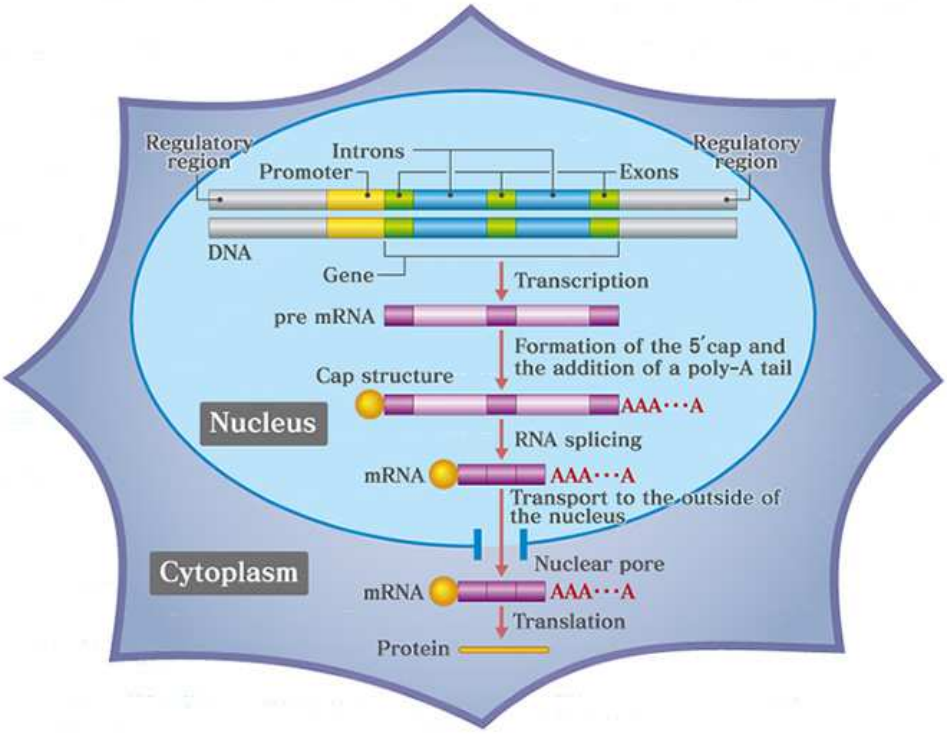


Fig. 1. Gene expression (http://csls-text.c.u-tokyo.ac.jp/active/04_03.html)

Translation initiation includes events that lead to positioning of 80S ribosome at the start codon of mRNA. Translation rates are primarily regulated at initiation level involving large number of initiation factors (Macdonald, 2001). The amount of mRNA available for translation can be changed at different steps of RNA maturation. Two families of proteins (the RNA binding proteins and RNA helicases) determinate the fate of pre-mRNAs and mRNA by regulating steps from transcription to translation (Mazzucotell et al., 2008). Translation control is critical in regulating wide range of process in cells form development, cell differentiation and proliferation to regulation of metabolic pathways. It is also important for protection of cell from external effects (Garcia-Sanz et al., 1998).

Transcription and translation regulation mechanisms are till now described in literature quite detail, but there is still only few data of post-transcriptional and post-translational regulation mechanism. Due to the fact that large number of RNA molecules is being synthesis in the cell, the precise post-transcriptional regulation is necessary to control the activity and location of produced RNA molecules. This mechanism is controlled by RNA-binging proteins (Halbeisen et al., 2007). Post-transcriptional regulations of gene expression occur at the levels of pre-messenger RNA (mRNA) processing (capping, splicing, and polyadenylation), mRNA stability, and mRNA translation (Floris et al., 2009). The last level of gene expression control is the post-translational regulation. This step is responsible for controlling the levels of protein activity. Post-translation protein modifications are

important for clinical research. There are few hounded of described post-translation modification; the most common are phosphorylation, ubiquitination, glycosylation, *S*-nitrosylation, proteolysis and methylation (Egorina et al., 2008).

3. Gene regulatory networks

When talking about biochemical network they can be divided into three groups: (i) metabolic network-describing chemical transformations between metabolites, (ii) protein networks (signaling networks)-describing protein-protein interaction and (iii) gene networks- describing relationships between genes (Brazhnik et al., 2002, Schlitt & Brazma, 2005). Key differences between regulatory and metabolic networks are listed in the Table 2.

Network feature	Metabolic networks	Regulatory networks
Structure	Hazard stochiometry	Qualitative statements
Evolutionary conservation	Enzyme sequences highly conserved across species	Limited conversion of *cis* regulatory sites between closely related species
Malleability	Fixed structure in terms of the substrates that a particular enzymes can process	Adjustable structure, because of the possibility that mutations in the *cis* regulatory sites change binding specificity
Level of biochemical characterization	Fairly complete understanding of most subsystems in microbial organisms	Most subnetworks have not been well characterized even in microbial model organisms
Modelling approaches	Quantitative constraint-based models can be constructed at the genome-scale	Quantitative models can be currently constructed only on a small scale; qualitative discrete network models can be used to study large networks
Role of noise	Relatively small because of the high concentrations of metabolites involved in most reactions	Possibly significant in determining both structural features of the network and the overall response of the network to a stimulus

Table 2. Differences between regulatory and metabolic networks (Herrgård et al., 2004)

Gene regulatory networks regulate the expression of thousands of genes. It can be sad that gene regulatory networks are maps of the interactions between regulatory gene products and their *cis* regulatory elements (gene and gene products interact and form networks), as well between signaling ligand and their receptor. So, basic functional unit of gene regulatory network is promoter region of a gene or operon which contains *cis* regulatory binding site for transcription factors, The location of binding sites and affinity of transcription factors determinate the level of gene expression (Herrgård et al., 2004) (Fig. 2).

Gene regulatory maps display flow of regulatory information throughout embryogenesis (Hinman et al., 2009). Gene network analysis provides many important information:

1. gene network provides information to help annotate genome
2. it helps to uncover the biochemical network in a cell
3. it provides new idea to treat some diseases (Liu et al., 2006).

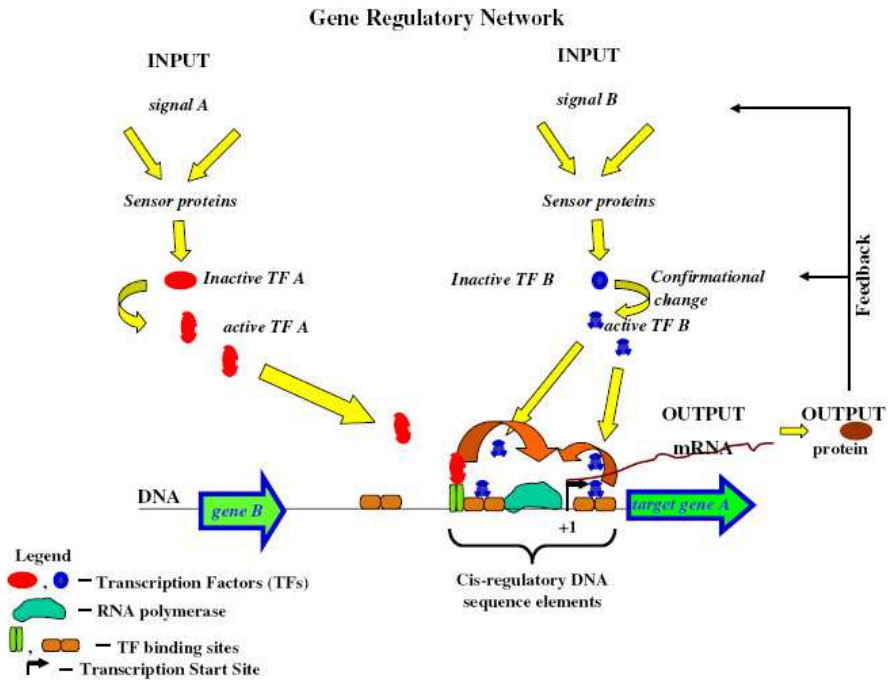


Fig. 2. Genomic view of gene regulatory network. From genomic perspective transcriptional regulation can be presented as an interplay between *cis*-regulatory elements and different transcription factors (Janga & Collado-Vides, 2007)

The activity of functional genes is influenced by few factors: transcriptional factors and cofactor that effects transcription, by degradation of proteins and transcripts and by post-translational modifications (Hecker et al., 2009). The idea of gene regulatory network is to describe dependence between molecules included in gene activity. Gene regulatory network is composed of nodes (representing genes proteins or metabolites) and edges (representing molecular interactions) (Hecker et al., 2009). Identification of gene regulatory networks is based on deterministic models of gene expression (Cinquemani et al., 2008).

The architecture of gene regulatory networks arise directly form DNA sequences of the genome and representation of gene regulatory networks must have specific emphasis on predicted DNA inputs and it has to be viewable at a number of different levels (Longabaugh et al., 2008). Identifying gene networks from large-scale dataset measurements is a difficult computational and experimental problem (Tegnér & Björkegren, 2006).

4. Mathematical modelling of gene regulatory network

As mentioned before, gene regulatory networks are becoming more and more usefully tool for analysis and understanding organization within cells and their dynamics (Crombach & Hogeweg, 2008). To better understand the complex process in gene regulatory networks, mathematical models of those systems have been developed. Mathematical models are very useful for predicting the effect of nonlinear interactions (Smolen et al., 2000) and can provide insight into systems understanding of regulation of processes in the cell (Zak et al., 2005). Gene regulatory networks are modelled as networks composed of nodes representing genes, proteins or metabolites and edges representing molecular interactions (protein-protein, DNA-protein or relationships between genes) (Hecker et al., 2009). The biggest problem in a field of mathematical modelling of gene regulatory networks is still in development of model based on experimental data because it is very difficult to defining the quality of available experimental data. There are many approaches for defining gene regulatory networks identification; in the most general manner we can defer unstructured and structured approach (Zak et al., 2005). In unstructured modelling approach there is assumption that every gene regulates every other gene. Using additional domain knowledge it is possible to develop structured model. Subcellular structure, nuclear connectivity and dynamical model structure have to be taken into consideration when developing structured model (Fig 3).

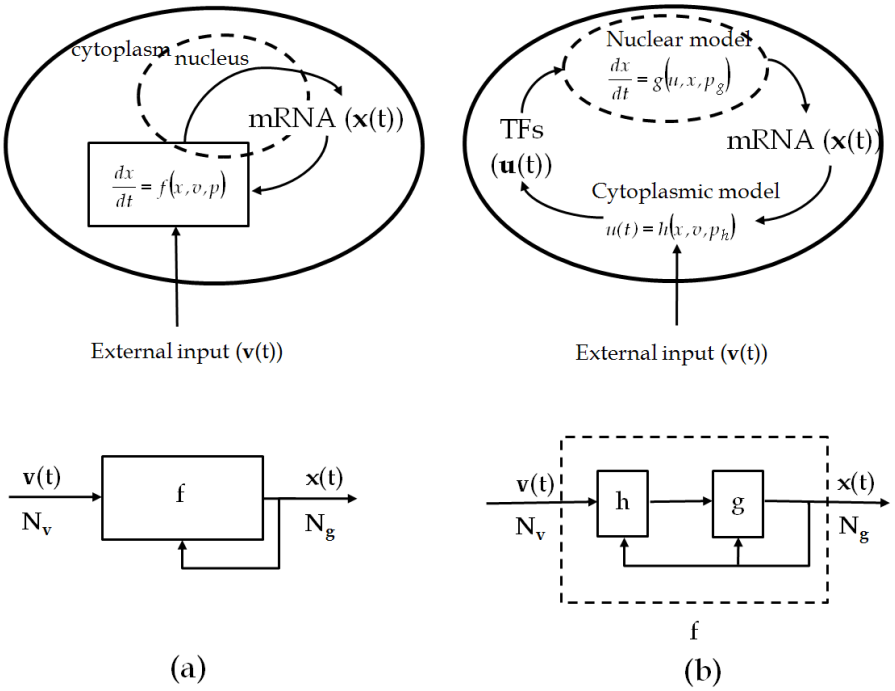


Fig. 3. (a) Unstructured and (b) structured gene regulatory network modelling (Zak et al., 2005)

Mathematical sciences can contribute to biology in field of models diversity. Different types of cell are developed as a consequence of the gene activity which is under control of gene regulatory network (Fig. 4) (Mochizuki, 2008).

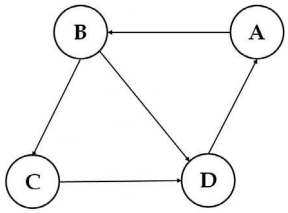


Fig. 4. Example of gene regulatory network (Mochizuki, 2008)

When developing model two facts have too be taken into account: (i) gene expression levels are regulated by the combined action of multiple gene products, (ii) the number of measurements is relatively small compared to the number of measured genes and measures noise has to be taken into account (van Someren et al., 2002). According to Schlitt & Brazma, 2005 gene networks models can be divided into four groups according to increasing level of detail in the models: (i) part lists, (ii) topology models, (iii) control logic models, (iv) dynamic models.

All mentioned approaches face the same two problems which make the automatic discovery of gene networks form experimental data far form trivial (van Someren et al., 2002). The first is statistical robustness and the second biological interpretation of the results (how to differ regulation form co-expression and indirect regulation form direct regulation) (Lulli & Romauch, 2009). When talking about statistical robustness the focus is the fact that high-dimensionality problem cusses the hypothetical models to be highly susceptible (number of microarray experiments is usually much smaller that number of genes included into network) (Chan et al., 2008).

4.1 Parts list

The first step in developing gene regulatory network is construction of a part list of the components included into network (Hu et al., 2010). High-throughput genome sequencing project have made available complete genomic lists of many organism (Alm & Arkin, 2003). Those lists include genes, transcriptional factors, promoters, binding sites and many other molecules important for functioning of gene network (Schlitt & Brazma, 2007).

4.2 Topology models

After defining the components of the gene networks, the next step of the modelling of the gene regulatory network is definition of the connections between nodes (definition of the edges). Development of network topology includes decision about genes are included into the networks, which acts as inhibitors or activator of transcription (Mendes et al., 2003). The different topology classes of networks (regular lattice, small-world, random networks…) are consequence of different ways how large sets of elements are connected (Gonçalves & Costa, 2008). Network growth model is present in Fig. 5. To quantitatively describe a network topology at minimum three metrics are employed:

1. clustering coefficients - for node i in a network with k_i edges connecting it to the nearest neighbours, the clustering coefficient is defined with Eq. 1.

$$C_i = \frac{2n}{k_i(k_i - 1)} \tag{1}$$

were n represents the number of edges between nearest neighbours. C_i can have numerical values between 0 and 1. When C_i=0 node is linked to disconnected group, and when C_i=1 node is connected to interlinked group.
2. network diameter – is defined as the smallest number of the links by which starting from one node another node can be reached
3. degree distribution – is probability $P(k)$ that a node has k links (Lukashin et al., 2003).

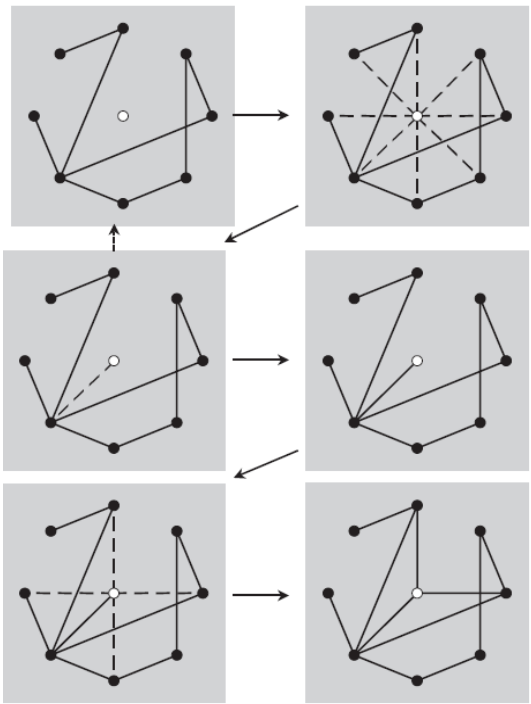


Fig. 5. Network growth model (Lukashin et al., 2003)

Ciliberti et al., 2007 analysed relationship between robustness and network topology for millions of networks with different topologies. Results showed that significantly different network architecture can show the same gene expression patterns. It was also noticed that some networks are highly robust to gene expression noise and mutations whereas some are quite fragile. Crombach & Hogeweg, 2008 analysed the evolution of gene regulatory networks. Their results showed that interplay between long term evolution process and short term gene regulation dynamics leads to increase in efficiency of crating adapted offspring.

4.3 Control logic models

After defining the network topology, the next step in development of gene regulatory network is analysis of the rules of the interaction between the network elements (Schlitt and Brazma, 2007). Transcriptional-regulatory systems is based on the presence of transcription factor binding sites of genes which are responsible for receiving temporal regulatory input signals; sequential logic model (SML) can be used for description of trans-activation and temporal mRNA expression profiles (Yeo et al., 2007). SML technique can ensure detail insight into gene regulation and it can ensure systematically analysis of the dynamic transcriptional inputs.

4.4 Dynamic models

The nodes in gene network population of genes, proteins and other regulatory molecules. There can be from few to few thousands of copies of those molecules in cell. Components of the gene regulatory networks can be changed in response to internal and external stimuli. It is important to include those interactions into network; this is possible using dynamic modelling approach. Dynamic modelling frameworks are usually classified along two axes: continuous versus discrete (describes the level of detail of node state) and deterministic versus stochastic (in view of uncertainties and variability of the transfer functions) (Albert, 2007). Dynamic models can also be divided into quantitative (base on system of ordinary differential equations) and qualitative models (piecewise linear differential system) (Chaouiya, 2007).

4.4.1 Boolean models

Boolean networks describe the state of genes with binary (ON/OFF) variables. Dynamic behaviour of each variable is governed by Boolean function (Albert, 2004). Although Boolean networks allow the analysis of the dynamics of the gene regulatory networks, they ignore the effect of genes at intermediate levels (Xu et al., 2007). Boolean networks have been intensely investigated as models for genetic control in cells. In those networks, each gene represents the node, and as mentioned before each node has two states ON (producing the protein) or OFF (there is no protein production). The biological basis for development of Boolean netwok as a model of gene regulatory network lies in the fact that during regulation of functional states the cell exhibits switch-like behaviour; this form of behaviour ensures the movement of cell from one state to another (Shmulevich et al., 2002). In the network there are links between nods (one node has impact on the other) (Pomerance et al., 2009). The Boolean networks have ability to contain very large number of nodes but are very crude in their approximation in biology (Karlsson & Hörnquist, 2007). In Boolean network form (Fig.6.), the genome is presented by set of binary variables, $g_1, g_2 \ldots g_N$. The expression of each gene changes with time according to Eq.2.:

$$g_n(t+1) = F_n\left(g_{n_1}(t), g_{n_2}(t) \ldots . g_{n_{k_n}}(t)\right) \tag{2}$$

where F_n is Boolean function constructed according to the inhibition or activation nature of the regulators. According to Balleza et al, 2008 if $F(g_1,g_2)$ is the function of two regulators g_1 and g_2 than function F can be in one of the following forms: $F(1,1)=1$, $F(1,0)=1$, $F(0,1)=0$ and $F(0,0)=1$. Regulatory phrase for $F=1$ is activator and for $F=0$ inhibitor.

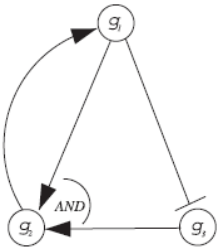


Fig. 6. Boolean network of three entities (Steggles et al., 2007)

Continuous models give more realistic description of the process, but development of those models requires large amounts of experimental data. As mentioned before in Boolean models at each time point the gene state depends on the state of the gene regulators at previous time step (Giacomantonio & Goodhill, 2010).

Some modification of traditional Boolean gene regulatory network models can be found in literature. One of them is temporal Boolean network. The difference between those two network models is in the fact temporal Boolean network allows the state of gene at time *t+1* depends on state of genes at times *t*, *t-1*,….., *t-(T-1)*(Silvescu & Honavar, 2001). Another approach is propped by Shulevich et al, 2002; probabilistic Boolean network. Probabilistic Boolean network includes properties of Boolean networks (rule-based dependence between genes), but due to the probabilistic nature this approach is suitable for systematic study of regulatory networks.

4.4.2 Petri net models

Petri net theory provides graphical notation with mathematical background. A Petri net is directed, bipartite and labelled graph which is build of four parts: (i) palces, denoted with circle representing biological compounds (metabolites), (ii) transitions, denoted with black rectangle, representing biochemical reactions between metabolites (iii) arcs, denoted with arrows and (iv) tokens denoted by black rectangle (Fig.7.) (Steggles et al., 2007).

As mentioned before, places represent resource of the system and can contain movable objects (tokens). Tokens represent quantitative unit of compounds. Transitions correspond to events that can change the state of the resources. Arcs (arcs label corresponds to stoichiometric number in reaction equation). Places represent resource of the system, and transitions correspond to events that can change the state of the resources. Arcs connect places to transitions (Chaouiya, 2007) and are allowed only between places and transitions and vice versa, never between places or between transitions (Koch et al., 2005).

According to Steggles et al., 2006, it is possible to develop gene regulatory network model based on Petri net starting from Boolean network. The idea was to use logic minimization to extract Boolean terms representing gene network and then to translate this into Petri net structure; the resulting Petri net model is capable to correctly capture dynamic behaviour of gene networks.

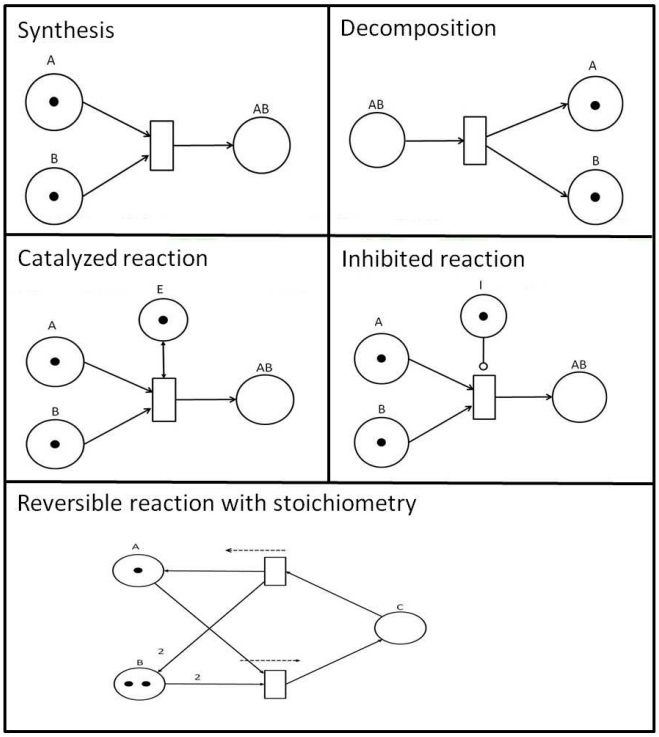


Fig. 7. Petri net modelling of different reactions (Chaouiya, 2007)

4.4.3 Difference and differential equation models

Using ordinary differential equations for representing gene regulatory networks concentrations of proteins, mRNAs and other molecules are presented as continuous time variables (Polynikis et al., 2009). Flexibility of ordinary deferential equations allows the description of complex relations between components of the net. Differential equations can describe complex dynamic behaviour like oscillations, cyclical patterns, multistationary and switch-like behaviour (Gebert et al., 2007). According to Hecker et al., 2009 the dynamic of gene regulatory networks can be described with (Eq.3.):

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x, p, u, t) \tag{3}$$

were $x(t) = (x_1(t).....x_n(t))$ represents gene expression vector of genes for 1 to n, f is function that describes the rate of change of variable x. p presents model parameter set and u external perturbation signals. Transcription and translation can be model using ordinary differential equations (Eq. 4-5):

$$\frac{\mathrm{d}r_i}{\mathrm{d}t} = F\left(f_i^R(p_1), f_i^R(p_2)....f_i^R(p_n)\right) - \gamma_i r_i \tag{4}$$

$$\frac{\mathrm{d}p_i}{\mathrm{d}t} = f_i^P(r_i) - \delta_i p_i \tag{5}$$

the function $f_i^R(p_j)$ is usually non-linear and describes the dependence of mRNA concentration on protein concentration. According to Hecker et al., 2009 ordinary differential equations for description of gene regulatory network can be divided into:

1. linear differential equations can used for description of gene expression kinetics (Eq.6)

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \sum_{j=1}^{N} w_{i,j} x_j + b_i u \tag{6}$$

Gebert et al., 2007 used developed model of pecewise linear differential equation for description of interaction between genes in regulatory networks; variables of the model were mRNA concentrations (Fig.8.).

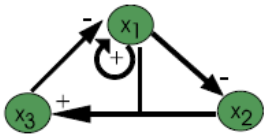


Fig. 8. Model of gene regulation (Gebert et al., 2007)

Model was based on assumption that regulation between genes can be described using piecewise linear differential equations (Eq 7-9).

$$\dot{x}_1 = k_{1,1} h^+\left(x_1, \theta_{1,1}, m_{1,1}\right) + k_{1,3} h^+\left(x_3, \theta_{1,3}, m_{1,3}\right) - \gamma_1 x_1 \tag{7}$$

$$\dot{x}_2 = k_{2,1} h^+\left(x_1, \theta_{2,1}, m_{2,1}\right) - \gamma_2 x_2 \tag{8}$$

$$\dot{x}_3 = k_{3,1,2} h^+\left(x_1, \theta_{3,1}, m_{3,1}\right) h^+\left(x_2, \theta_{3,2}, m_{3,2}\right) - \gamma_3 x_3 \tag{9}$$

were γ represents the degradation rate of mRNA and k are rate constants.
Wu et al., 2004 proposed method to model gene expression dynamic from measured time-course data including linear equations. Developed dynamic equations described the relationships between internal state variables and observation variables.

2. non-linear differential equations are used for describing complex dynamic behaviours. Comparing to linear models, identification of the non-linear differential equation model is computationally more intensive and it requires more data (Quian & Wang, 2007). The numerical representation of non-linear ODE model of gene regulatory network (Eq.10):

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = f_i\left(x_1, x_2, ..., x_n\right) + \nu_i \qquad i = 1, 2, ...N \tag{10}$$

where f_i represents the non-linear function which can be determinated from experimental data, it can be polynomial (Eq.11):

$$f_i = \sum_{j=1}^{L_i}\left[\left(w_{ij} + \mu_{ij}\right)\Omega_{ij}\left(x_1, x_2, ..., x_n\right)\right] \qquad i = 1,2,...N \tag{11}$$

where L_i is the number of terms in f_i, w_{ij} represent parameters that need to be estimated and $\Omega_{ij}(x_1, x_2, \ldots x_N)$ is the component of the nonlinear function.

Quian and Wang, 2007 developed gene regulatory network model including evolutionary algorithm and filtering approach; noise was modelled using nonlinear ordinary differential equations. Simulation showed the usage of proposed model on experimental data for microarray experiments.

Using set of ordinary differential equations for description of gene network, the inference of genetic networks is often defined as a function optimization problem to minimize the defences between gene expression levels obtained numerically and levels measured in experiments (Kimura et al., 2009). The problems that occurs when working with differential equation model are that those models include many parameters which have to be estimated form experimental data or obtained from literature. It also has to be taken into consideration that for complex differential equations analytical solution and analysis of the equations can be very complex.

4.4.4 Stochastic modelling

All cellular events depend on probabilistic collisions between molecules. Due to the fact that number of events occurring in the cells is not large and events are dependent, it is not possible to use deterministic rate equations for description of the gene network (gene expression is stochastic process (Paulsson, 2005). There are many important stochastic phenomena during the life time of the cell, like random fluctuations that initiate transcription, spontaneous jumps in mRNA or protein concentrations (Rosenfeld, 2007). Study of stochastic properties in genetic systems involves formulation of molecular noise, formulation of approximation of these representations and development of computational algorithms capable for describing complexity of network dynamics (El Samad, et al., 2005).

According to Rosenfeld, 2007 for mathematical description of stochastic dynamics of gene regulatory networks two approaches can be used:

1. non-linear dynamics paradigm – treats the biochemical components included in gene expression regulation as continuous variables and describes their variations using non-linear differential equations
2. Markov process paradigm – due to the fact that some molecules included into gene expression regulation can occur in cell in very low concentrations they can not be treated as a continuous variables and their random fluctuations can be very high.

Stochastic modelling approach is mathematically represented with Eq.12:

$$p(x, t + \Delta t) = p(x, t + \Delta t) \cdot \left(1 - \sum_{j=1}^{m} \alpha_j \Delta t\right) + \sum_{j=1}^{m} \beta_j \Delta t \tag{12}$$

where x represents the amount of molecules (state variable), $p(x,t)$ probability distribution. Assuming that $\Delta t \to 0$, the equation for stochastic representation of gene regulatory network is developed Eq. 13:

$$\frac{\partial p(x,t)}{\partial t} = \sum_{j=1}^{m} \left(\beta_j - \alpha_j p(x,t) \right) \tag{13}$$

4.4.5 Finite state linear models

Methodology of finite state linear modelling (FSLM) was developed by Bramza & Schlitt, 2003; it combines discrete and continuous aspects of gene regulation in structured way. Model was developed on few assumptions: (i) gene activity is defined by state of transcription binding sites in promoter region, (ii) each binding site can be in one of the finite number of states, (iii) active gene produces substance with rate dependant on activity level, (iv) state of binding site depends on concentrations of transcription factors. The continuous parts of the model consist of the state of the proton concentrations. As mentioned before it also includes Boolean-type model gene regulation (each gene and each binding site can have only two sates; ON or OFF). Bramza & Schitt, 2003 used finite state linear model for construction of biological network of λ-phage (Fig.9.)

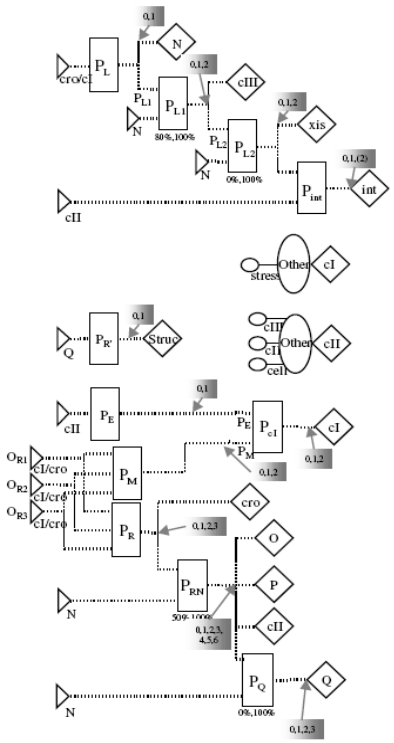


Fig. 9. Gene network of λ-phage (Bramza & Schitt, 2003)

Ruklisa et al., 2005 used previously described FSLM model for testing some theoretical properties of the model: (i) what kind of network dynamic could be modelled using this framework, (ii) is it possible to describe chaotic network dynamics and some others. A series of experiments were performed to estimate the regularity of behaviour of random networks; networks were simulated in 10000 steps and results show that FSLM models can be suitable for describing biological reality.

4.4.6 Hybrid models

Due to fact that boundaries between discreet and continuous model depend on the level of details included into the model there is an attempt to develop the models that could include both approaches (Schlitt & Bramza, 2007). Matsuno & Doi, 2000 proposed hybrid Petri net model for presentation of gene regulatory networks of λ-phage. Hybrid Petri net is an extension of Petri net that has continues and discrete elements and can be easily used for protein or mRNA concentration. Another approach to development of hybrid model is present by Crudu et al., 2009. They proposed unified framework for hybrid simplification of the Markov models of stochastic gene network dynamics. It was shown that those simplified models describe with good accuracy the stochastic properties of the gene networks and can be used for multi-scale biochemical systems.

5. Conclusion

Gene expression can be regulated on few levels. Gene regulatory networks are defined as collections of DNA segments in cells which interact with each other. Construction of gene regulatory networks is first step in biological analysis. It is very import to understand and explain the dynamic of gene regulatory networks. To explain and understand those complex biochemical systems different mathematical models have been developed. Techniques of mathematical modelling defer in level of details. Each modelling technique has its advantages and disadvantages and that has to be taken into consideration when developing mathematical model, because proposed model has to provide good insight into gene regulation process and be useful for prediction of some possible mutations or any other change.

6. Future direction section

When modelling gene regulatory networks the fact that model describes only some properties has to be taken into consideration. So there is always open question how real the developed model can be (Schlitt & Bramza, 2007). Using new molecular methods large amount of data can be collected ensuring the better insight into process in the cell. Including all this information in the model more detail model can be developed. When talking about mathematical modelling of gene regulatory networks the neural networks have been used lately for modelling (Lee & Yang, 2008; Xu et al., 2008). For example Knott et al., 2010 presented approach to model gene regulatory networks as non-liner system using artificial neural network. There is also idea in developing synthetic networks. All described approaches have one goal developing simple model which would describe the process in the cell; so the future direction of modelling of gene regulatory networks would be in funding the way how to reduce the complexly of biological systems and to preserve the model functionality.

7. Acknowledgment

This work was supported by Ministry of Science, Education and Sport of Republic of Croatia grant MZOŠ 0589.

8. References

Alm, E. & Arkin, A.P. (2003) Biological network. *Current Opinion in Structural Biology*, Vol. 13, No. 2, (April, 2003), pp. 193-202, ISSN 0959-440X

Albert, R. (2004) Boolean modelling of genetic regulatory networks. *Lecture Notes in Physics*, Vol.650, pp. 459-481, ISSN 0075-8450

Albert, R. (2007) Network inference, analysis, and modelling in systems biology. *The Plant Cell*, Vol. 19, (November 2007), pp.3327-3338, ISSN 1532-298X

Balleza, E.; Alvarez-Buylla, E.R., Chaos, A., Kauffman, S., Shmulevic, I. & Aldana, M. (2008) Critical dynamics in genetic regulatory networks: examples from four kingdoms. *Plos One*, Vol. 3, No. 6, (June 2008) pp. 1-10, ISSN 1932-6203

Berg, J.M.; Tymoczko, J.L., Stryer, L. & Clarke, N.D. (2001) Biochemistry, W.H. Freeman and Company, ISBN 0-7167-4954-8

Bramza, A. & Schlitt, T. (2003) Reverse engineering of gene regulatory networks: a finite state linear model. *Genome Biology*, Vol. 4, No. 6, (April 2003) pp. P5, ISSN 1465-6906

Brazhnik, P.; de la Fuente, A. & Mendes, P. (2002) Gene networks: how to put the function in genomics. *Trends in Biotechnology*, Vol. 20, No. 11, (November 2002) pp. 467-472, ISSN 0167-7799

Bruggeman, F.J. & Westerhoff, H.V. (2007) The nature of systems biology. *Trends in Microbiology*, Vol. 15, No. 1, (January, 2007), pp. 45-50, ISSN 0966-842X

Chan, Z.S.H.; Havaukkala, I., Jain, V., Hu, Y. & Kasabov, N. (2008) Soft computing methods to predict gene regulatory networks: An integrative approach on time-series gene expression data. *Applied Soft Computing*, Vol.8, pp. 1189-1199, ISSN 1568-4946.

Chaouiya, C. (2007) Petri net modelling of biological networks. *Briefing in Bioinformatics*, Vol.8, No.4, (July 2007), pp. 210-219, ISSN 1477-4054

Ciliberti, S.; Martin, O.C. & Wagner, A. (2007) Robustness can evolve gradually in complex regulatory gene networks with varying topology. *Plos Computational Biology*, Vol. 3, No. 2, (February 2007), pp. 0164-0173, ISSN 1553-734X

Cinquemani, E.; Milias-Argeitis, A., Summers, S. & Lygeros, J. (2008) Stochastic dynamics of genetic networks: modeling and parameter identification. *Bioinformatics*, Vol. 24, No. 23, pp. 2748-2754, ISSN 1471-2105

Crombach, A. & Hogeweg, P. (2008) Evolution of evolvability in gene regulatory networks. *Plos Computational Biology*, Vol.4, (July 2008), pp. 1-13, ISSN 1553-7358

Crudu, A.; Debusschu, A. & Radulesc, O. (2009) Hybrid stochastic simplifications for multiscale gene networks. *BMC Systems Biology*, Vol.3, pp. 89-114, ISSN 1752-0509.

Day, D.A. & Tuite, M.F. (1998) Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *Journal of Endocrinology*, Vol. 157, pp.361-371, ISSN 1479-6805

Dilão, R. & Muraro, D. (2010) A software tool to model genetic regulatory networks. Applications to the modelling to threshold phenomena and of spatial patterning in *Drosophila*. *Plos One*, Vol. 5, No. 5 (May 2010), pp. e1074, ISSN 1932-6203

Egorina, E.M., Sovershaev, M.A. & Østerud, B. (2008) Regulation of tissue factor procoagulant activity by post-translational modifications. *Thrombosis Research,* Vol. 12, No. 6, pp. 831-837, ISSN 0049-3848

El Samad, H.; Khammash, M., Petzold, L. & Gillespie, D. (2005) Stochastic modelling of gene regulatory networks. *International Journal of Robust and Nonlinear Control*, Vol. 15, pp. 691-711, ISSN 1049-8923

Floris, M.; Mahgoub, H., Lanet, E., Robaglia, C. & Benoit, M. (2009) Post-transcriptional regulation of gene expression in plants during abiotic stress. *International Journal of Molecular Sciences,* Vol. 10, pp. 3168-3185, ISSN 1422-0067

Garcia-Sanz, J.A.; Mikulits, W., Livingstone, A., Lefkovits, I. & Müllner, E.W. (1998) Translational control: a general mechanism for gene regulation during T cell activation. *The Journal of the Federation of American Societies for Experimental Biology,* Vol. 12, No.3, (March 1998), pp.299-306, ISSN 1530-6860

Gebert, J.; Radde, N. & Weber, G.-W. (2007) Modelling gene regulatory networks with piecewise linear differential equations. *European Journal of Operational Research*, Vol. 181, pp. 1148-1165, ISSN 0377-2217

Halbeisen, R.E.; Galgano, A., Scherrer, T. & Gerber, A.P. (2007) Post-transcriptional gene regulation: From genome-wide studies to principles. *Cellular and Molecular Life Sciences*, Vol. 65, No. 5, (March 2008), pp. 798-813, ISSN 1420-9071

Hinman, V.F.; (2009) Evolution of gene regulatory network architectures: Examples of subcircuit conservation and plasticity between classes of echinoderms

Hecker, M.; Lambeck, S., Toepfer, S., van Someren, E. & Guthke, R. (2009) Gene regulatory network inference: Data integration in dynamic models-A review. *BioSystems,* Vol.69, No.1, (April 2009), pp. 86-103, ISSN 0303-2647

Herrgård, M.J.; Covert, M.W. & Palsson, B.Ø. (2004) Reconstruction of microbial transcriptional regulatory networks. *Current Opinion in Biotechnology*, Vol. 15, No.1, (February 2004), pp. 70-77, ISSN 0958-1669

Hu, J.; Wan, J., Hackler Jr., L., Zack, D.J. & Qian, J. (2010) Computational analysis of tissue-specific gene networks: application to murine retinal functional studies. *Bioinformaics,* Vol. 26, No.18, (September 2010), pp. 2289-2297, ISSN 1471-2105

Janga, S.C. & Collado-Vides, J. (2007) Structure and evolution of gene regulatory networks in microbial genome. *Research in Microbiology*, Vol.158, No.10 (December 2007), pp. 787-794, ISSN 0923-2508

Janky, R.; van Helden, J., Madan Babu, M. (2009) Investigating transcriptional regulation: from analysis of complex networks to discovery of *cis*-regulatory elements. *Methods*, Vol. 48, No. 3, (July 2009), pp. 277-286, ISSN 1046-2023

Karlsson, F. & Hörnquist, M. (2007) Order or chaos in Boolean gene networks depends on the mean fraction of canalizing functions. *Physica A: Statistical Mechanics and its Application*, Vol. 384, No.2 (October 2007), pp. 747-757, ISSN 0378-4371

Kimura, S.; Nakayama, S., Hatakeyama, M. (2009) Genetic network inference as a series of discrimination tasks. *Bioinformatics*, Vol. 25, No. 7 pp.918-925 ISSN 1471-2105

Knott, S.;Mostafavi, S. & Mousavi, P. (2010) A neural network based modelling and validation approach for identifying gene regulatory networks. *Neurocomputing*, Vol. 73, No. 13-15, (August, 2010), pp. 2419-2429, ISSN 0925-2312

Koch, I.; Junker, B.H. & Heiner, M. (2005) Application of Petri net theory for modelling and validation of the sucrose breakdown pathway in the potato tuber. *Bioinformatics*, Vol. 21, No. 7, pp. 1219-1226, ISSN 1471-2105

Lee, W.-P. & Yang, K.-C. (2008) A clustering-based approcah for interfernig rucerrent neural netwoks as gene regulatory networks. *Neurocomputing*, Vol. 71, No. 4-6, (January 2008), pp. 600- 610, ISSN 0925-2312

Lewin, B. (2004) Genes, Pearson Prentoce Hall, ISBN 0-13-123924-4

Liu, T.-F.; Sung, W.-K. & Mittal, A. (2006) Model gene network by semi-fixed Bayesian network. *Expert Systems with Application*, Vol.30, No.1, (January 2006), pp. 42-49, ISSN 0957-4174

Longabaugh, W.J.R.; Davidson, E.H. & Bolouri, H. (2009) Visualization, documentation, analysis, and communication of large-scale gene regulatory networks. *Biochimica et Biophysica Acta,* Vol. 1789, No. 4, (April 2009), pp. 363-374, ISSN 0006-3002

Lukashin, A.V.; Lukashev, M.E. & Fuchs, R. (2003) Topology of gene expression networks as revealed by data mining and modelling. *Bioinformatics*, Vol. 19, No. 15, (October 2003) pp. 1909-1916, ISSN 1471-2105

Lulli, G. & Romauch, M. (2009) A mathematical program to refine gene regulatory networks. *Discrete Applied Mathematics*, Vol. 157, pp. 2469-2482, ISSN 0166-218X

Macdonald, P. (2001) Diversity in translational regulation. *Current Opinion in Cell Biology*, Vol. 13, No.3, (June 2001), pp. 326-331, ISSN 0955-0674

Matsuno, H. & Doi, A. (2000) Hybrid Petri net representation of gene regulatory networks. *Biocomputing 2000, Proceeding of the Pacific Symposium*, pp. 338-349, ISBN 981-238-598-3, Hawaii, USA, January 23-27, 2000

Mazzucotelli, E.; (2008) Abiotic stress response in plants: When post-transcriptional and post-translational regulations control transcription. *Plant Science*, Vol. 174, No. 4, (April 2008), pp. 420-431, ISSN 0168-9452

Médigue, C. & Moszer, I. (2007) Annotation, comparison and databases for hundreds of bacterial genomes. *Research in Microbiology*. Vol. 158, No. 10 (December 2007), pp. 724-736, ISSN 0923-2508

Mendes, P.; Sha, W. & Ye, K. (2003) Artificial gene networks for objective comparison of analysis algorithms. *Bioinforamtics*,Vol.19, No.2, pp. 122-129, ISSN 1460-2059

Mochizuki, A. (2008) Structure of regulatory networks and diversity of gene expression patterns. *Journal of Theoretical Biology*. Vol. 250, No. 2 (January 2008), pp.307-321, ISSN 0022-5193

Paulsson, J. (2005) Models of stochastic gene expression. *Physics of Life Reviews*, Vol.2, No.2, (June 2005), pp. 157-175, ISSN 1571-0645

Polynikis, A.; Hogan, S.J. & di Bernardo, M. (2009) Comparing different ODE modelling approaches for gene regulatory networks. *Journal of Theoretical Biology*, Vol. 261, pp. 511-530, ISSN 0022-5193

Pomerance, A.; Ott, E., Girvan, M. & Losert, W. (2009) The effect of network topology on the stability of discrete state models of genetic control. *PNAS*, Vol. 106, No. 20, (May 2009), pp. 8209-8214, ISSN 1091-6490

Qian, L. & Wang, H. (2007) Inference of genetic regulatory networks by evolutionary algorithm and H_ filtering. *14th IEEESP Workshop on Statistical Signal Processing SSP 07*, pp. 21-25, ISBN 9781424411979

Reed, J.L.; Famili, I., Thiele, I. & Palsson, B.O. (2006) Towards multidimensional genome annotation. *Nature Reviews Genetics*, Vol.7, (February 2006), pp. 130-141, ISSN 1471-0056

Rosenfeld, S. (2007) Stochastic cooperativity in non-linear dynamics of gene regulatory networks. *Mathematical Biosciences*, Vol. 210, No. 1, (November 2007), pp. 121-142, ISSN 0025-5564

Ruklisa, D.; Bramza, A. & Viksna, J. (2005) Reconstruction of gene regulatory networks under finite state linear model. *Genome Informatics,* Vol. 16, No. 2, pp. 225-236, ISSN 0919-9454

Rust, A.G.; Mongin, E., Birney, E. (2002) Genome annotation techniques: new approaches and challenges. *Drug Discovery Today*, Vol. 7, No. 11, (May 2002), pp. s70-s76, ISSN 1359-6446

Silvescu, A. & Honavar, V. (2001) Temporal Boolean Network Models of Genetic Networks and their Inference from Gene Expression Time Series. *Complex Systems*, Vol. 13, pp. 61-78, ISSN 0891-2513

Schlitt, T. & Brazma, A. (2005) Modelling gene networks at different organisational levels. *FEBS Letters*, Vol.579, No.8, (March 2005), pp. 1859-1866, ISSN 0014-5793

Shmulevich, I., Doughtery, E.R., Kim, S. & Zhang, W. (2002) Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks. *Bioinformatics,* Vol. 18, No. 2, pp. 261-274, ISSN 1460-2059

van Someren, E.P.; Wessels, L.F.A., Backer, E. & Reinders, M.J.T. (2002) Genetic network modeling. *Pharmacogenomics*, Vol. 3, No. 4, pp.507-525, ISSN 1462-2416

Smolen, P.; Baxter, D.A. & Byrne, J.H. (2000) Mathematical modelling of gene networks. *Neuron*, Vol. 26, (June 2000), pp. 567-580, ISSN 0896-6273

Steggles, L.J.; Banks, R., Shaw, O. & Wipat, A. (2007) Qualitatively modelling and analysing genetic regulatory networks: a Petri net approach. *Bioinformatics*, Vol.23, No.3, (March 2007), pp. 336-343, ISSN 1460-2059

Steggles, L.J.; Banks, R. & Wipat, A. (2006) Modelling and analysing genetic networks: From Boolean networks to Petri nets. *Lecture Notes in Computer Sciences*, Vol.42, (October 2006), pp. 127-141, ISSN 0302-9743

Strizh, I.; Joutchkov, A., Tverdokhlebov, N. & Golitsyn, S. (2007) Systems biology and grid technologies: Challenges for understanding complex cell signaling networks. *Futire Generation Computer Sytems*, Vol.23, No.3 (March, 2007), pp. 428-434, ISSN 0167-739X

Tegnér, J. & Björkegren, J. (2007) Perturbations to uncover gene networks. *Trends in Genetics*, Vol. 23, No. 1, (January 2007), pp. 34-41, ISSN 0168-9525

Wilczynski, B. & Furlong, E.E.M. (2010) Challenges for modelling global gene regulatory networks during development: insight from *Drosophila*. *Developmental Biology.* Vol. 320, No. 2, (April 2010), pp. 161-169, ISSN 1471-213X

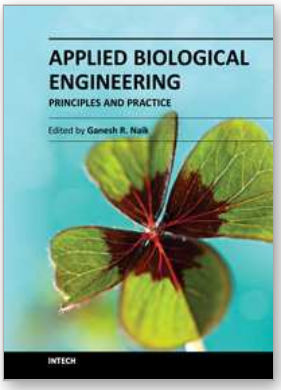
Wu, F.X.; Zhang, W.J. & Kusalik, A.J. (2004) Modeling gene expression from microarray expression data with state-space equations. *Biocomputing 2004, Proceeding of the Pacific Symposium*, pp. 581-592, ISBN 981-238-598-3, Hawaii, USA, January 6-10, 2004

Xu, R.; Venayagamoorthy, G.K. & Wunsch, D.C. (2007) Modelling of gene regulatory netwoks with hybrid differential evolution and particle swarm optimization. *Neural Networks*, Vol. 20, pp. 917-927, ISSN 0893-6080

Yakunin, A.F.; Yee, A.A., Savchenko, A., Edwards, A.M., Arrowsmith, C.H. (2004) Structural proteomics: a tool for genome annotation. *Current Opinion in Chemical Biology*, Vol. 8, No. 1 (February 2004), pp. 42-48, ISSN 1367-5931

Yeo, Z.X.; Wong, S.T.,Vel Arjunan, S.N. Piras, V., Tomita, M., Selvarajoo, K., Guiliani, A. & Tsuchiya, M. (2007) Sequential logic model deciphers dynamic transcriptional control of gene expressions. *Plos One*, Vol. 2, No. 8 (August 2007), pp. 1-18, ISSN 1932-6203

Zak, D.E.; Vadigepalli, R., Gonye, E.G., Doyle, F.J., Schwaber, J.S. & Ogunnaike, B.A. (2005) Unconventional systems analysis problem in molecular biology: a case study in gene regulatory network modelling. *Computational and Chemical Engineering,* Vol.29, No.3, (February 2005), pp. 547-563, ISSN 0098-1354



Applied Biological Engineering - Principles and Practice
Edited by Dr. Ganesh R. Naik

ISBN 978-953-51-0412-4
Hard cover, 662 pages
Publisher InTech
Published online 23, March, 2012
Published in print edition March, 2012

Biological engineering is a field of engineering in which the emphasis is on life and life-sustaining systems. Biological engineering is an emerging discipline that encompasses engineering theory and practice connected to and derived from the science of biology. The most important trend in biological engineering is the dynamic range of scales at which biotechnology is now able to integrate with biological processes. An explosion in micro/nanoscale technology is allowing the manufacture of nanoparticles for drug delivery into cells, miniaturized implantable microsensors for medical diagnostics, and micro-engineered robots for on-board tissue repairs. This book aims to provide an updated overview of the recent developments in biological engineering from diverse aspects and various applications in clinical and experimental research.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821