# THE MATHEMATICAL MODEL OF GENETIC REGULATION OF PYRIMIDINE BIOSYNTHESIS IN *ESCHERICHIA COLI*

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***Motivation and Aim:*** Development of an *in silico* cell as a computer resource for the modeling and analysis of intracellular processes is a topical problem of systems biology and bioinformatics. Within this direction, it is necessary to develop mathematical models of the genetic regulation of cell metabolic pathways, in particular, the regulation of pyrimidine biosynthesis. It is of interest, because its decision permits us to control the metabolism of the bacterial cell. The aim of this work was to discover characteristics of functioning and regulation of pyrimidine biosynthesis in *Escherichia coli* cell using mathematical modeling methods.

***Methods:*** The gene network of pyrimidine biosynthesis was reconstructed using the *GeneNet* system [1]. The method of generalized Hill functions [2] and MGSgenerator [3] was used to create a base of elementary mathematical models (BEMM) of enzymatic reactions and genetic elements of this process. «Mathematica 5.2» was used for constructing the model of pyrimidine biosynthesis and perfoming *in silico* experiments.

***Results:*** The reconstructed gene network of pyrimidine biosynthesis contains 106 objects (protein, mRNA, genetic elements and metabolites), which cohere in integrated system of 132 elementary events (reaction, regulatory interaction). The base of elementary mathematical models contains 72 elementary subsystems composed of 9 enzymatic reactions, 23 reactions of protein degradation, 9 metabolite outflow reactions, 10 metabolite inflow reactions and 21 reactions of genetic regulation. Parameters of elementary models were selected according to kinetic characteristics of subsystems functioning that were measured experimentally. Basing on BEMM the mathematical model was developed, describing dynamics of pyrimidine nucleotide biosynthesis in *E. coli* cell. The model was adapted to experimental data. Stationary concentrations of proteins and metabolites were calculated. The concentrations were in consistence with that in stationary phase of *E.coli* **(Table 1).**

**Table 1.** Stationary concentrations of substances [molecules per cell] in stationary phase.

| Monomers of enzymes | Monomers of transcription factors (TFs) | Metabolites |
|---|---|---|
| PyrG_monomer = 1225 | SoxR_monomer = 59 | Orotate (OROA) = 147 |
| Ndk_monomer = 2401 | Crp_monomer = 713 | Aspartate (ASP) = 5,1E+5 |
| PyrH_monomer = 3600 | OxyR_monomer = 64 | Glutamine (Gln) = 1,94E+5 |
| PyrF_monomer = 1225 | Fis_monomer = 75 | Dihydroorotate (DOROA) = 180 |
| PyrE_monomer = 1225 | Fur_monomer = 3600 | Carbamoyl phosphate (CAP) = 3,85E+3 |
| PyrD_monomer = 754 | PurR_monomer = 544 | Orotidine-5'-phosphate (OMP) = 31 |
| PyrC_monomer = 754 | PepA_monomer = 110 | Uridine triphosphate (UTP) =1,3E+6 |
| PyrI_monomer = 724 | ArgR_monomer = 490 | Carbonic acid gas (CO2) = 7,58E+6 |
| PyrB_monomer = 724 | NusA_monomer = 43 | Uridine diphosphate (UDP) = 5,9E+4 |
| CarB_monomer = 2510 | SoxS_monomer = 25 | Cytidine triphosphate (CTP) = 3,8E+5 |
| CarA_monomer = 2510 | IhfA_monomer = 13470 | Guanosine triphosphate (GTP) = 8,1E+5 |
| | IhfB_monomer = 13470 | Adenosine triphosphate (ATP) = 1,1E+6 |
| | | Uridine monophosphate (UMP) = 5,6E+4 |
| | | N-carbamoyl-L-aspartate (CAASP) = 680 |
| | | 5'-phosphoribosile pyrophosphate (PRPP) = 5.3E+4 |

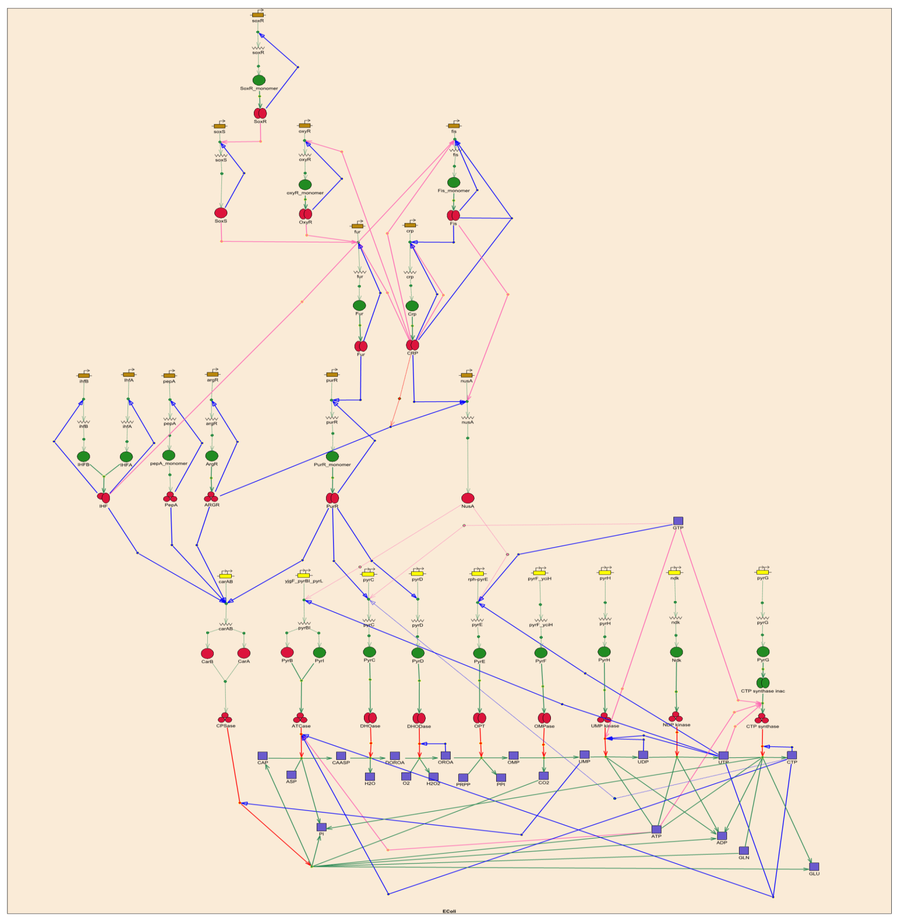

**Fig. 1** The gene network of pyrimidine biosynthesis in *Escherichia coli* cell was reconstructed using the GeneNet system [1].

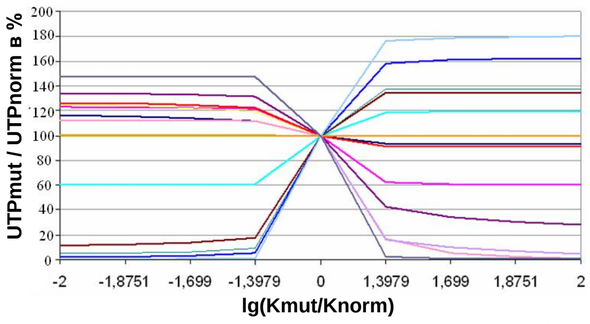


**Fig. 2** Alteration of the UTP steady-state concentration in a cell in dependence on change of protein degradation constants in the gene network of pyrimidine nucleotides biosynthesis. The abscissa axis is logarithm of the relation of the altered constant to the constant at the stationary phase. The ordinate axis corresponds to the relation of the altered UTP concentration to the stationary phase concentration of UTP.

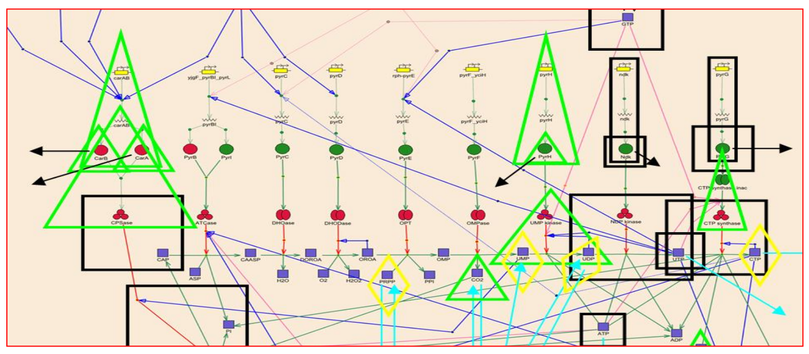

**Fig. 3** A part of the gene network of pyrimidine nucleotides biosynthesis with sensitivity of UTP stationary concentrations in a cell to mutational changing of parameters signed. Alteration of constants,corresponding to defined biological processes, that are differentially colored, influence on the stationary phase concentration of UTP and vary it in different limits: red – no more than 25%; black - from 25% to 75%; green - within 75-100% limit; yellow – > 100%.

To reveal limiting processes and to analyze the dynamic of pyrimidine nucleotide biosynthesis in different environment and internal conditions we investigated the behavior of steady-state of the model at changing values of its parameters. Altering values of individual parameters one by one we observed the alteration of concentrations of target products CTP and UTP. Fig. 2 shows curves of UTP steady-state concentration change in a cell in dependence on changing all protein degradation constants. To achieve these results 2880 calculations were done. Summarized results are partially shown in fig.3. In this figure a part of pyrimidine nucleotide biosynthesis gene network is shown with sensitivity of stationary UTP level in a cell to alteration of parameters values signed. About 60% of parameters change stationary concentration of UTP (CTP) pyrimidine nucleotides no more than 25%, what can be interpreted as high resistance of pyrimidine nucleotide metabolism. The resistance is caused by unlimited reactions and negative feed-back loops in the genetic network. According to results of analysis on sensitivity of the model to parameters variation, changing less then 4% of parameters leads to increased yield of products for more than 100%.

The *in silico* experiments were performed to discover the influence of outside regulators on levels of UTP and CTP in a stationary phase of a cell. The biological prerequisite of this goal was that fact that during normal cell development under aerobic conditions highly reactive molecules are formed, damaging DNA, proteins, lipids and affecting on the membrane transport. Outside agents that influence on formation of highly reactive molecules may be paraquat (PQ) and NO·. That rise the question on investigation of pyrimidine nucleotide dynamic under oxidative stress.

The solution of this goal within the bounds of created model is complicated by independence of CTP and UTP levels on PQ and NO· concentrations. Regulation involves the net of interactions of regulatory proteins (TF). It is known that affectivity of expression of SoxS TF gene is under positive control by PQ and NO· under oxidative stress conditions. As a result synthesized SoxS triggers the stress respond cascade also regulating *fur* expression, the TF of which is inhibiting expression of TF of PurR that is in turn the regulator of three enzymes of pyrimidine nucleotide biosynthesis. It was the reason why the base of submodels was supplemented with a mathematical model describing the subsystem of soxRS expression regulation in dependence of NO· and PQ concentrations. Developed the model of the elementary subsystem describing the influence of NO· and PQ on soxRS operon expression level (Fig. 5) we made numerical calculations of CTP and UTP synthesis in dependence of the level of soxRS operon expression. Fig. 4 illustrates theoretical computations of stationary concentrations of target substances in dependence on PQ and NO· concentrations. The antiphase effect is detected, when the concentration of one substance rises another one decreases. This effect due to the negative feed-back loop, when CTP as a product inhibits it's own synthesis (Fig. 1).

Addition of PQ cause monotonous increase fashion of UTP concentration change and monotonous decrease for CTP concentration (Fig, 4A). Under increasing volumes of NO· curves are bell-shaped (Fig. 4B). Difference in curves fashions can be explained by increasing of the soxRS expression level while increasing PQ concentrations (Fig. 5A), as a result UTP level increases and CTP decreases. Contrary increase in volumes of NO· results in increased soxRS operon expression till it reaches a maximal level of expression and then decrease of SoxR and SoxS proteins concentration can be observed forming a bell-shaped curve.

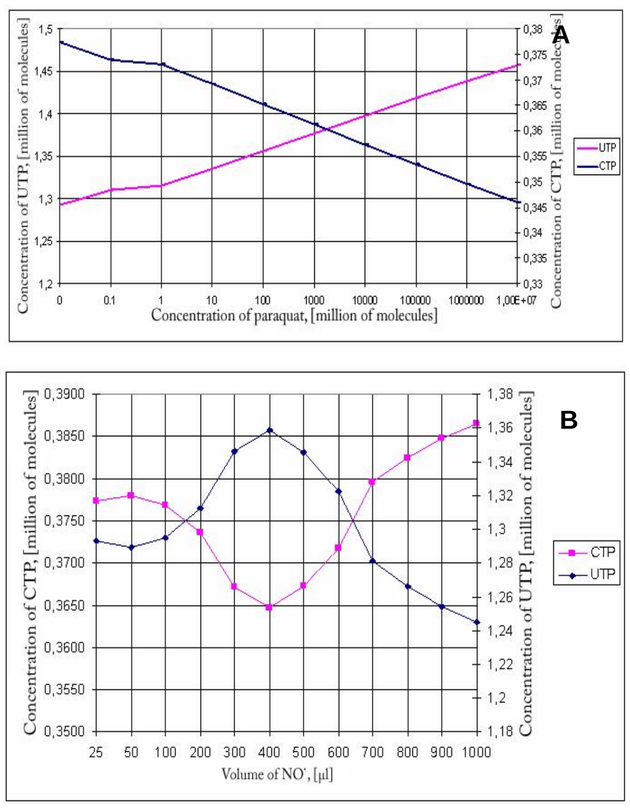


**Fig. 4** Theoretical calculations of target products CTP and UTP steady-state concentrations in dependence on concentration of PQ (A) and different volumes of NO· (B).

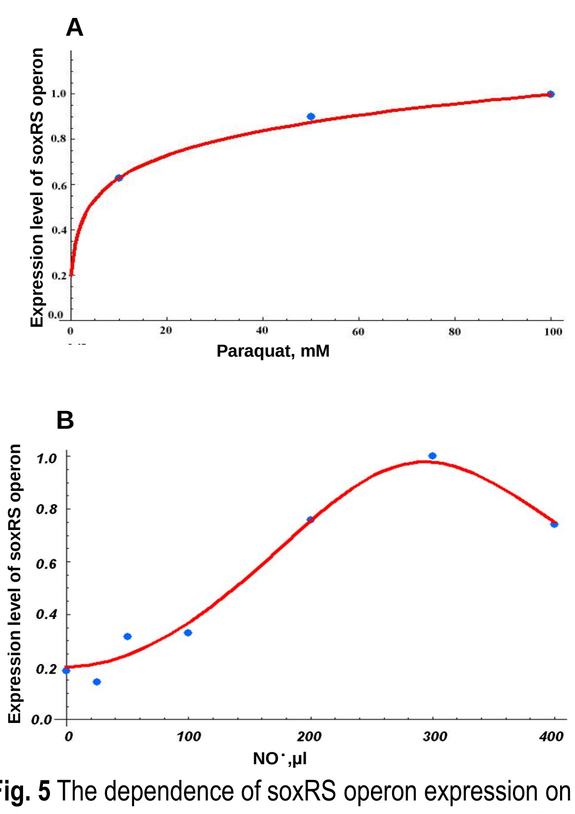


**Fig. 5** The dependence of soxRS operon expression on PQ concentration (A) and different volumes of NO· (B). Points correspond to experimental data from [*Nunoshiba et al.,1993, 1992*], solid lines correspond to results of the model, obtained with optimal set of parameters.

***Conclusion:*** Using the *GeneNet* computer technology the gene network of pyrimidine nucleotide biosynthesis was reconstructed. The model of genetic regulation of pyrimidine nucleotide biosynthesis was created. Using the method of generalized Hill functions the problem of optimal values of parameters adjustment was solved. Quantitative fitness of numerically computed concentrations of system elements (proteins, metabolites) and fluxes rates of enzymatic reactions to experimental data corresponding to stationary phase of *E.coli* growth was achieved. The analysis of sensitivity of the model to parameters variation revealed that variation of about 60% of parameters leads to alteration of steady-state pyrimidine nucleotide concentrations no more then 25% that is interpreted as high resistance of pyrimidine nucleotide metabolism to alteration of biochemical reaction rates. The resistance is caused by unlimited reactions and negative feed-back loops in the genetic net. According to results of analysis of sensitivity of the model to parameters variation, changing less than 4% of parameters, related to subsystems of PRPP, UMP, UDP inflow and PRPP, CTP, UDP, CO2 outflow, resulted in increased of products yield more than 100%. Obtained results can be used to solve the problem of producer construction. Using the mathematical model the accumulation of target products (UTP and CTP) was calculated in dependence on nitric dioxide (NO·) and paraquat (PQ) concentrations in environment. It was shown that concentrations of UTP and CTP are in antiphase to each other due to the presence of negative feed-back loop, regulating the enzymatic conversion of UTP to CTP.

***References:***

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