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Impact of negative feedbacks on *de novo* pyrimidines biosynthesis in *Escherichia coli*

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Background Previous studies aimed to investigate the metabolism of endogenous nucleoside triphosphates in synchronous E. coli cell cultures demonstrated an existence of the self-oscillatory mode of the functioning of pyrimidines and purines biosynthesis. Authors of these studies associated the dynamic behavior with cell division. The metabolic system theoretically has an internal oscillatory potential due to feedback mechanisms orchestrating its dynamics. The question of whether the nucleotide biosynthesis system has its own oscillatory circuit is still open. To solve this issue, an integral mathematical model of pyrimidine biosynthesis was developed, which explores all experimentally verified negative feedbacks in the regulation of enzymatic reactions, data on detection under in vitro conditions. The scheme is presented in Fig.1.

Methods The search for solution sequences was carried out using STEP package algorithms (Fadeev, Gainova, 1996; Fadeev et al., 2006). The model analysis for dynamic modes was conducted with a combination of methods, DEEP (Kozlov, Samsonov, 2011) and Bayesian Spectrum Analysis (BaSAR) (Granqvist et al., 2012). A manual parameter fitting approach was used because values of almost all model parameters were known as well as a program implementation of the DEEP method was employed (Kozlov, Samsonov, 2011).

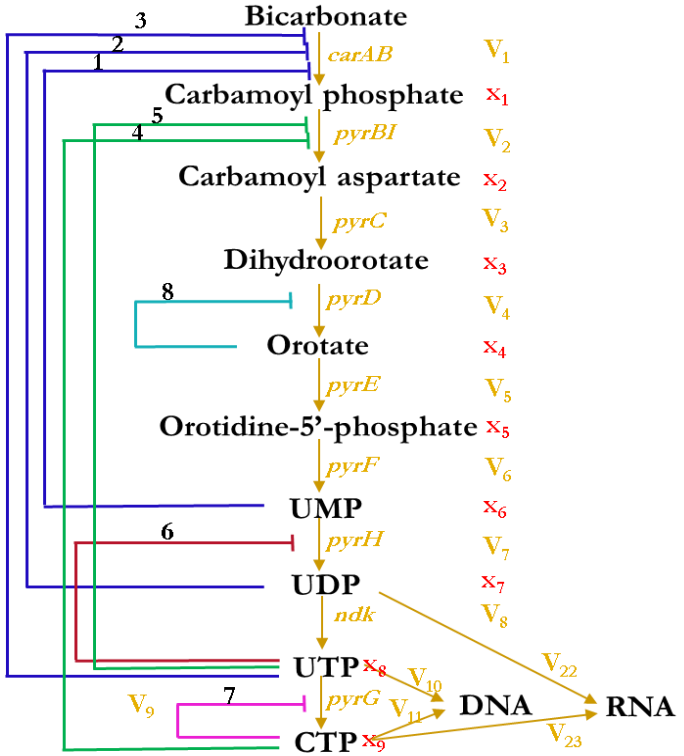

Fig. 1. A scheme of the regulation of metabolic pathway of the pyrimidines biosynthesis *de novo* in *E.coli.*
Directed arcs – direction of the reaction; while lines with blunt end are negative feedbacks regulating enzyme activities by biosynthetic products The numbers above them represent the order number of the regulatory relationships in the model analysis. Blue color – regulation of carbamoylphosphate synthetase (*carAB*); green– of aspartate transcarbamoylase (*pyrBI*); cyan– of dihydroorotate dehydrogenase (*pyrD*); red – of UMP kinase (*pyrH*); pink – of CTP synthetase (*pyrG*). V_j (j = 1,…,9) – the rate of corresponding enzymatic reaction; V_{10}, V_{11}, V_{22}, V_{23} – drain rates; X_j (j=1,…,9) – the model variables (metabolite concentrations).

Integrated mathematical model (1) of the pyrimidines biosynthesis

The mathematical model was constructed on the basis of the submodels of enzymatic reactions constituting the whole metabolic pathway of the pyrimidines biosynthesis in *E.coli*. The integrated model (1) is a system of nine ordinary differential equations describing rates of the concentration changes for metabolites produced and consumed in the pyrimidines biosynthesis pathway. The model variables are concentrations of metabolites: x_1 (CAP), x_2 (CAASP), … x_9 (CTP). Other substances included in elementary models (ATP, IMP,…) are considered as external compounds, concentrations of which are set as the model parameters and do not change in numerical simulations. The model equations (1) are shown on the right for reactions 1, 2, 4, 7 and 9, controlled by negative feedback mechanisms.

$$V_1=k_{1f}\times E_1\times\frac{\frac{BC}{K_{m_{BC}}}\times\frac{GLN}{K_{m_{GLN}}}\times\left(\frac{ATP}{K_{m_{ATP_1}}}\right)^2}{\left(1+\frac{BC}{K_{m_{BC}}}\right)\times\left(1+\frac{GLN}{K_{m_{GLN}}}\right)\times\left(1+\frac{ATP}{K_{m_{ATP_1}}}\right)^2}\times\frac{\left(1+\delta_{orn}\times\left(\frac{orn}{k_{orn_1}}\right)^{horn}\right)}{\left(1+\left(\frac{orn}{k_{orn_1}}\right)^{horn}\right)}\times\frac{\left(1+\delta_{IMP}\times\left(\frac{IMP}{k_{IMP_1}}\right)\right)}{1+\left(\frac{x_6}{K_{UMP_1}}\right)^{hUMP_1}+\left(\frac{IMP}{K_{IMP_1}}\right)+\left(\frac{x_7}{K_{UDP_1}}\right)^{hUDP_1}+\left(\frac{x_8}{K_{UTP_1}}\right)^{hUTP_1}}$$

$$\begin{cases}\frac{dx_1}{dt}=V_1-V_2\\ \frac{dx_2}{dt}=V_2-V_3\\ \frac{dx_3}{dt}=V_3-V_4\\ \frac{dx_4}{dt}=V_4-V_5\\ \frac{dx_5}{dt}=V_5-V_6\\ \frac{dx_6}{dt}=V_6-V_7\\ \frac{dx_7}{dt}=V_7-V_8-V_{15}\\ \frac{dx_8}{dt}=V_8-V_9-V_{10}\\ \frac{dx_9}{dt}=V_9-V_{12}-V_{11}\end{cases}$$

$$V_2=E_2\times k_{2f}\times\frac{\left(\frac{x_1}{K_{m_{CAP}}}\right)^{hCAP}}{1+\left(\frac{x_1}{K_{m_{CAP}}}\right)^{hCAP}}\times\frac{\left(\frac{asp}{K_{m_{asp_2}}}\right)^{h_{asp_{21}}}}{1+\left(\frac{asp}{K_{m_{asp_2}}}\right)^{h_{asp_{22}}}}\times\frac{1+\delta_{CTP_2}\times\left(\frac{x_9}{K_{CTP_2}}\right)+\delta_{ATP_2}\times\left(\frac{ATP}{K_{ATP_2}}\right)+\delta_{UTP_2}\times\left(\frac{x_8}{K_{UTP_2}}\right)}{1+\left(\frac{x_9}{K_{CTP_2}}\right)+\left(\frac{ATP}{K_{ATP_2}}\right)+\left(\frac{x_8}{K_{UTP_2}}\right)+\omega_2\times\left(\frac{x_9}{K_{CTP_2}}\right)\times\left(\frac{x_8}{K_{UTP_2}}\right)},$$

(1)

where

$$V_4=\frac{E_4\times\left(k_{4f}\times\frac{Q}{K_{m_Q}}\times\frac{x_3}{K_{m_{DOROA_4}}}-k_{4r}\times\frac{QH2}{K_{m_{QH2_4}}}\times\frac{x_4}{K_{m_{OROA_4}}}\right)}{\left(1+\frac{Q}{K_{m_Q}}+\frac{QH2}{K_{m_{QH2_4}}}\right)\times\left(1+\frac{x_3}{K_{m_{DOROA_4}}}\times\left(1+\frac{x_4}{K_{OROA_4}}\right)+\frac{x_4}{K_{m_{OROA_4}}}\right)},V_4=\frac{\left(k_{4f}\times\frac{x_3}{K_{m_{DOROA_4}}}-k_{4r}\times\frac{x_4}{K_{m_{OROA_4}}}\right)}{k_4\times\left(1+\frac{x_3}{K_{m_{DOROA_4}}}\times\left(1+\frac{x_4}{K_{OROA_4}}\right)+\frac{x_4}{K_{m_{OROA_4}}}\right)},$$

$$V_7=\frac{E_7\times k_{7f}\times x_6\times ATP}{\left(K_{m_{UMP_7}}\times\left(1+(1-r)\times\left(\frac{x_8}{K_{UTP_{72}}}\right)^{h_{UTP_7}}\right)+x_6\right)\times\left(K_{m_{ATP_7}}+ATP\right)}\cdot\frac{1}{1+r\times\left(\frac{x_8}{K_{UTP_{71}}}\right)^{h_{UTP_7}}}\times f_7,\ f_7=\frac{1+\delta_{GTP_7}\times\left(\frac{GTP}{K_{GTP_7}}\right)^{h_{GTP_7}}}{1+\left(\frac{GTP}{K_{GTP7}}\right)^{h_{GTP_7}}},$$

$$V_9=\frac{E_9\times k_{9f}\times(x_8)^{h_{UTP_9}}\times(ATP)^{h_{ATP_9}}}{\left(\left(K_{m_{UTP_9}}\right)^{h_{UTP_9}}\times\left(1+\frac{x_9}{K_{CTP_9}}\right)+(x_8)^{h_{UTP_9}}\right)\times\left(\left(K_{m_{ATP_9}}\right)^{h_{ATP_9}}+\left(1+\frac{ATP}{K_{ATP_9}}\right)\times(ATP)^{h_{ATP_9}}\right)}\times f_9,\ f_9=\frac{k_{0_9}+\delta_{GTP_9}\times\left(\frac{GTP}{K_{GTP_{91}}}\right)+\delta_{GTP_{91}}\times\left(\frac{GTP}{K_{GTP_{92}}}\right)^{h_{GTP_9}}}{1+\left(\frac{GTP}{K_{GTP_{91}}}\right)+\left(\frac{GTP}{K_{GTP_{92}}}\right)^{h_{GTP_9}}},$$

Dynamic modes: a numerical analysis of the model (1)

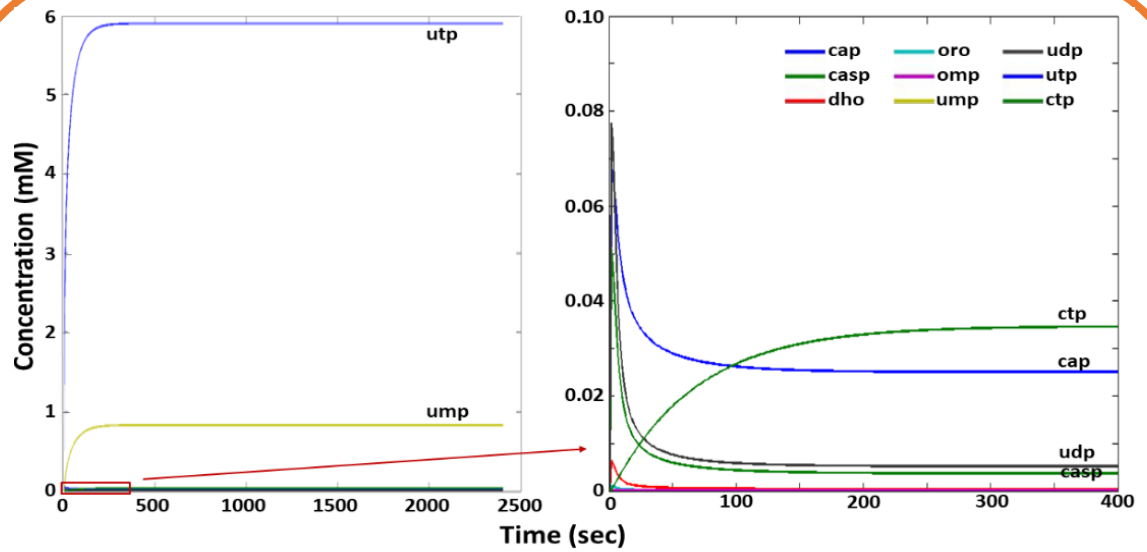

Fig. 2. The results of the model (1) simulation (1) manually fitted to the experimental data.

It is shown that the steady-state in the system of pyrimidines biosynthesis exists under the given set of the model parameters and a significant amount of only two metabolites (UMP and UTP) which are negative regulators of the system is observed.

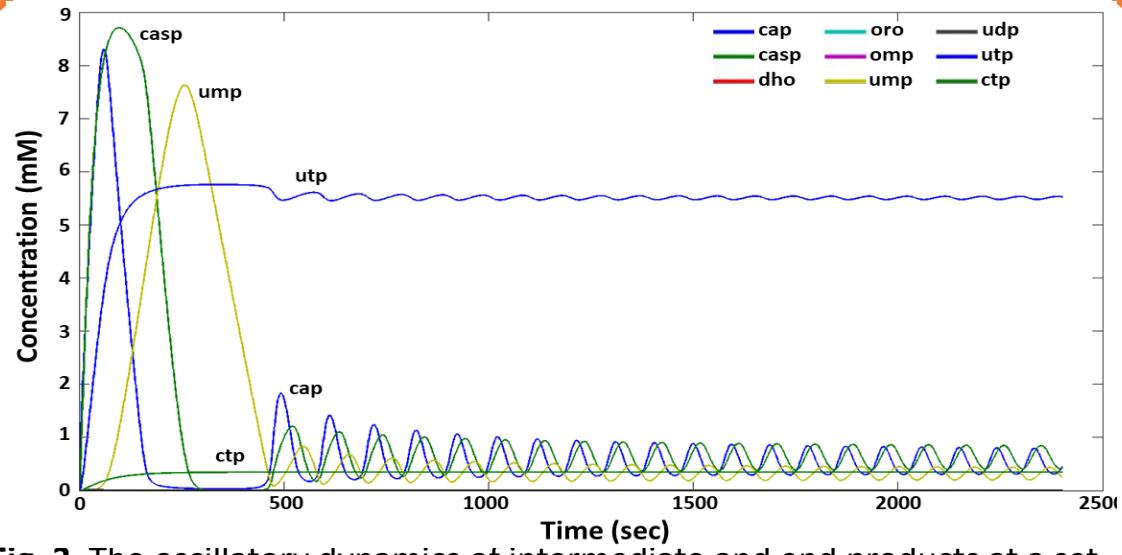

Fig. 3. The oscillatory dynamics of intermediate and end products at a set of the model parameters: $h_{UMP_1} = 2.1, k_2 = 0.0031, k_3 = 0.00351, k_9 = 0.000174$ и $r = 1$.

The oscillatory mode of the model at a set of the model parameters is shown. The key metabolic regulators of the mode switch are UMP and probably UTP the oscillatory dynamics in the biosynthesis of which via regulatory feedbacks 1, 3, 5 и 6 can impact the activity of at least three enzymes of the pyrimidine biosynthesis: carbamoylphosphate synthetase, aspartate transcarbamoylase and UMP kinase.

A numerical analysis of the model (1) by the parameter continuation method (Fadeev, Gainova, 1996) enabled us to identify a minimal set of parameters the values change of which have the most significant impact on the dynamic mode of the model and leads to oscillations in the system of pyrimidines biosynthesis. Among them are parameters characterizing the generalized efficiency of an enzyme functioning: aspartate transcarbamylase (k_2), dihydroorotase (k_3) and CTP synthetase (k_9) as well as a parameter of the nonlinear effect (h_{UMP_1}) of the UMP influence on the catalytic activity of carbamoylphosphate synthetase.

Dynamic modes: a numerical analysis of the model (1)

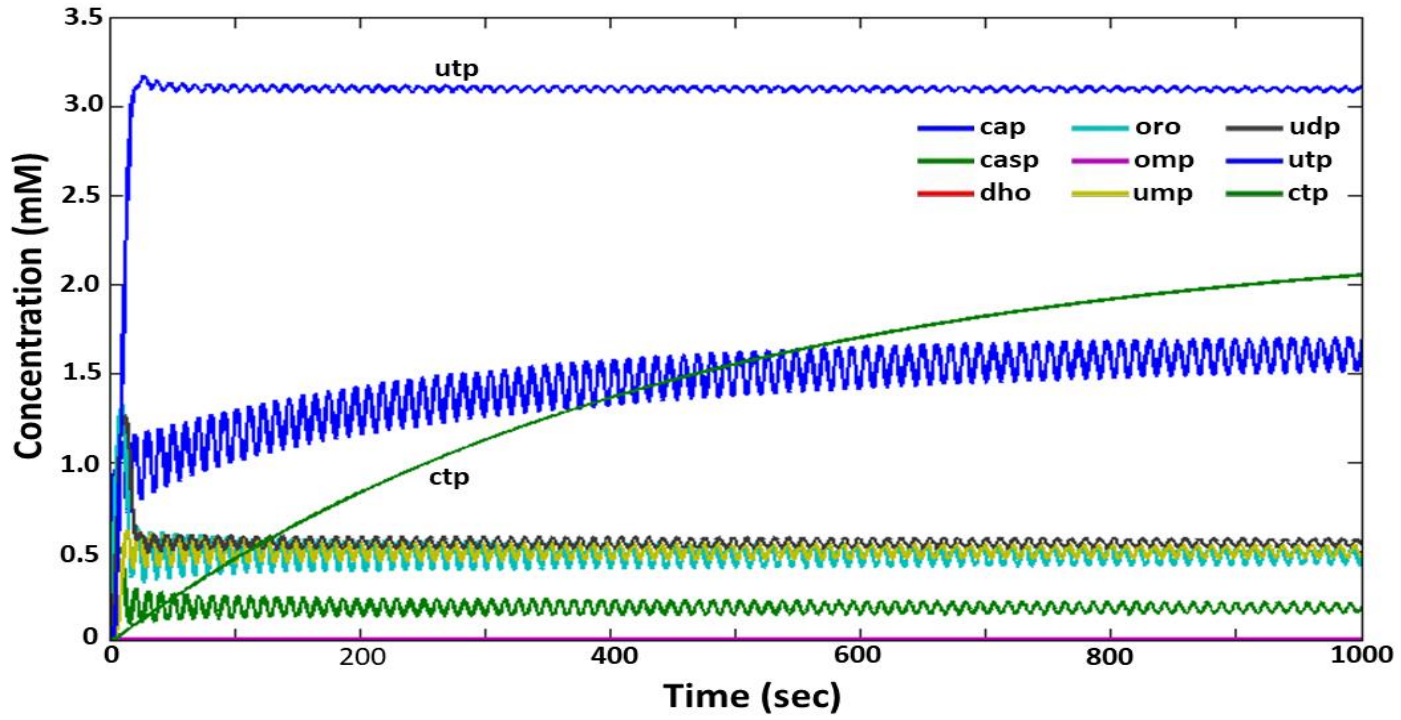

Fig. 4. The oscillatory mode of the system of pyrimidine biosynthesis was also obtained using DEEP method for the model calibration (Kozlov, Samsonov, 2011). The fitting procedure was simultaneously conducted for two experimental datasets. Parameter values: $h_{UMP_1} = 6, r = 0.28$.

It was demonstrated that the emergence of oscillations in the system depends on the balance of two model parameters: Hill coefficient h_{UMP_1} – nonlinear effect of UMP impact the activity of carbamoylphosphate synthetase and parameter *r*, the impact of uncompetitive mechanisms of the UTP inhibition in the regulation of UMP phosphorylation's enzymatic reaction.

The impact of negative feedbacks complexity on the dynamics of pyrimidines metabolism in *E. coli* cell considering two hypotheses on the mechanism of UTP inhibition of the UMP kinase activity – competitive and uncompetitive (parameter *r*)

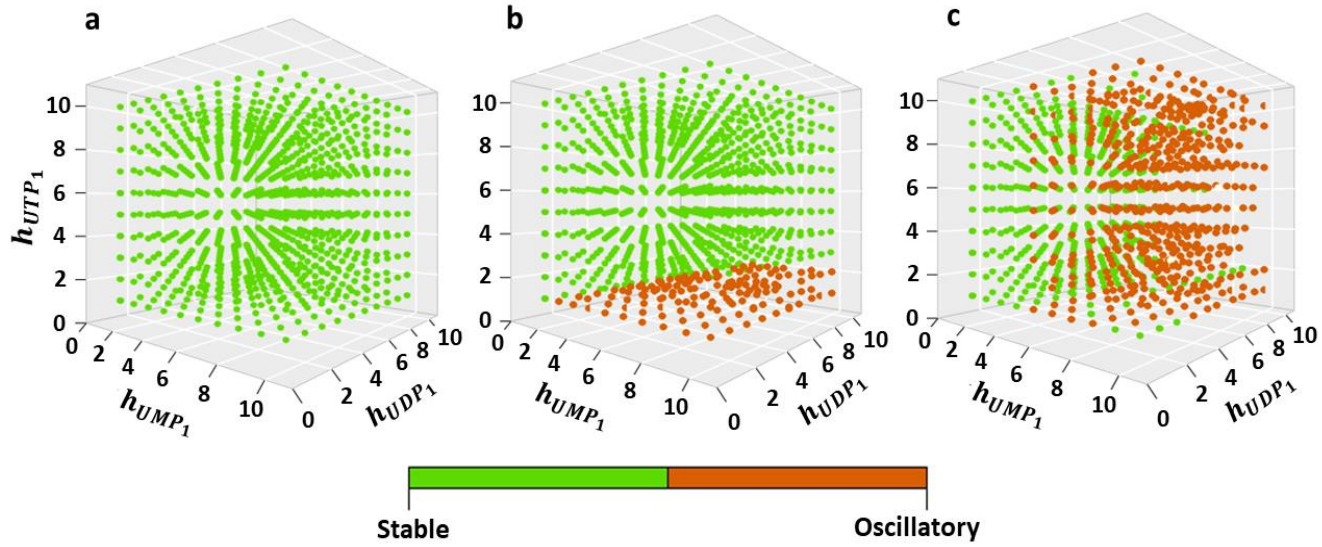

Fig. 5. The dynamic state of the metabolic system in the 3D space of Hill coefficients (h_{UMP_1},h_{UDP_1}, h_{UTP_1}).
(a) The model manually fitted to the data of Bennet et al., 2009; (b) the model for that the oscillation mode was manually identified at the parameters set ($h_{UMP_1} = 2.1, k_2 = 0.0031, k_3 = 0.00351, k_9 = 0.000174, r = 1$), (c) the model for that the oscillation mode was obtained by the DEEP method based on the calibration to two experimental datasets. Green points indicated a steady-state of the system, while orange ones are stable oscillations.

It is shown that the model version “a” does not have oscillatory mode independently of the parameter values in the range; models “b” and “c”– a probability of the oscillations emergence at uncompetitive mechanism (r=1) of the UTP inhibition of UMP phosphorylation (feedback 6) sharply increases compared to thecompetitive one.

Conclusions It was suggested that the system of pyrimidines biosynthesis in *E. coli* has its own oscillatory mode the potential of that substantially depends on the mechanisms of UMP kinase’s activity.