MATHEMATICAL MODELING OF NUCLEOTIDE BIOSYNTHESIS IN *ESCHERICHIA COLI*

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Motivation and Aim: The development of the ***in silico cell***, a bioinformatics resource for modeling, simulation and analysis of intracellular processes, is vital for systems biology. In this regard, the modeling of pyrimidine and purine nucleotide metabolism in *E. coli* cells is of not only purely scientific but also practical significance. In addition to the fact that nucleotides are components of DNA and RNA, ATP and GTP are involved in nearly all cell processes as phosphate and energy sources. Formulas on the base of various nucleotides are widely used in medicine and sports. Their industrial production, in particular, by transgenic producers, is still expensive. Mathematical modeling of processes and analysis of model behavior against altered genetic background provide an approach to the solution of pertinent fundamental and applied problems.

Methods and Algorithms: The construction of elementary models invoked the mass-action law, Michaelis–Menten equation, King–Altman method, and generalized Hill functions [1]. Metabolic system models were constructed as systems of ordinary differential equations describing global rates of variations in low-molecular-weight compound concentrations consumed or produced in the system to be modeled. The global rates were calculated from the law of summation of local rates described in the elementary models. Numerical calculations and model analysis were performed with STEP+ software [2].

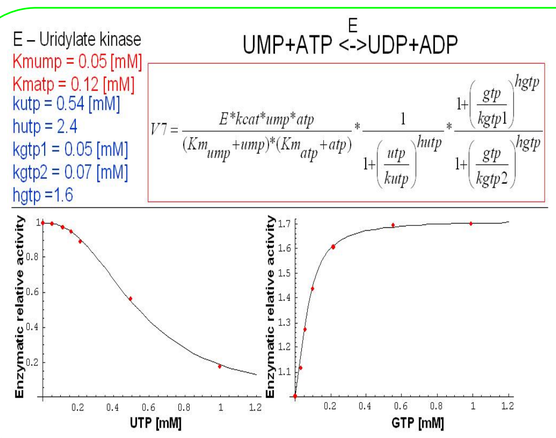

Fig.2. An elementary mathematical model describe the rate (V7) enzymatic phosphorylation reaction in the UMP pyrimidine pathway, depending on the concentration of UTP and GTP. Parameters marked with red color taken from the literature, blue - chosen on the basis of the kinetic curves of the article [*Serina_1995*].

Results: Basing on results from [3,4] elementary models of Vi (i=1, …, 22) enzymatic reactions velocities of pyrimiding biosynthesis (a Fig. 1A) and Vj (j=1, …, 27) –of biosynthesis purine (a Fig. 1B) are developed. One of 47 developed elementary models, describing velocity of UMP phosphorilation is shown in fig. 2. Biosynthesis models of pyrimidine (MPir) and purine (MPur) are presented in fig. 1C, D. It is numerically established that MPir functions in a mode of not fading self-oscillations (a Fig. 3A). It is revealed that duration of the fluctuation period is inversely proportional to doubling time of the cell. For a rapid divided cell the fluctuation period of substances concentration is equal to tens seconds, and for a slowly deviding cell – up to 20 minutes (a Fig. 3B). For MPur the stationary mode of functioning is observed only.

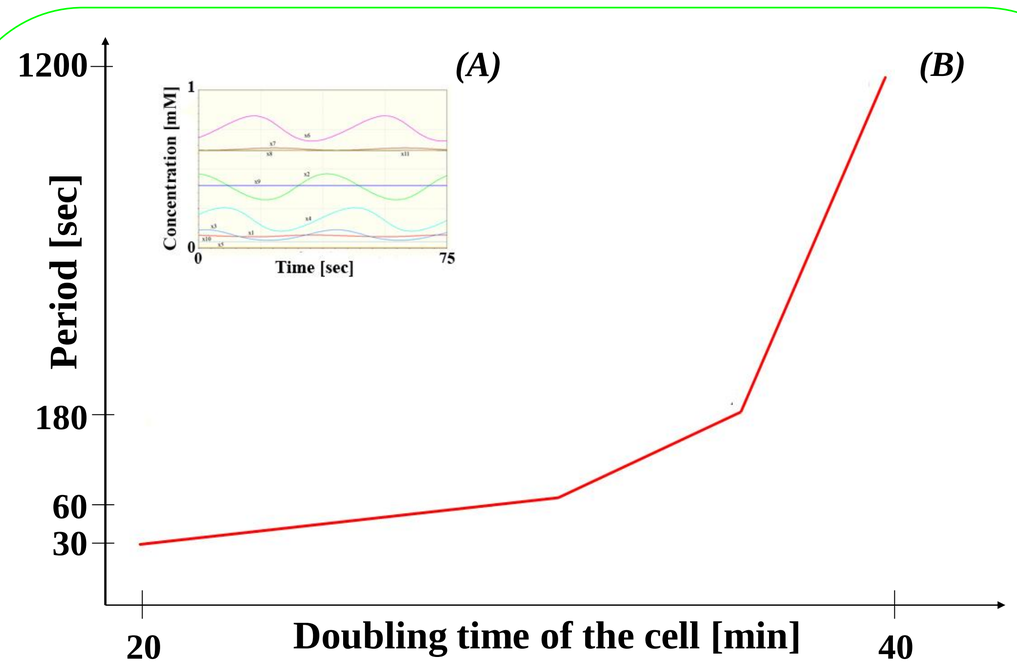

Fig.3. Correlation between doubling time and oscillation period of pyrimidine nucleotide and their precursor concentration

The continuation method on parameter [2] for M establishes parametrical borders of existence of self-oscillations (Fig. 4) and influence on them of 128 various combinations control loop is investigated.

It is shown that for all combinations containing control loop 1 (64 combinations) model M functions in an oscillatory mode that testifies to key value of a contour 1 for formation of the given mode.

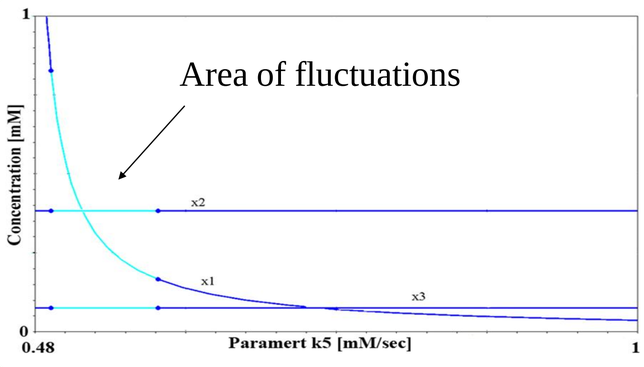

Fig. 4. Curves describing different concentration values of substances (for example, x1, x2, x3) at different values of parameter k5. Area of parameter value noted by blue color is responsible for a self-oscillation mode, and dark blue for a stationary solution mode.

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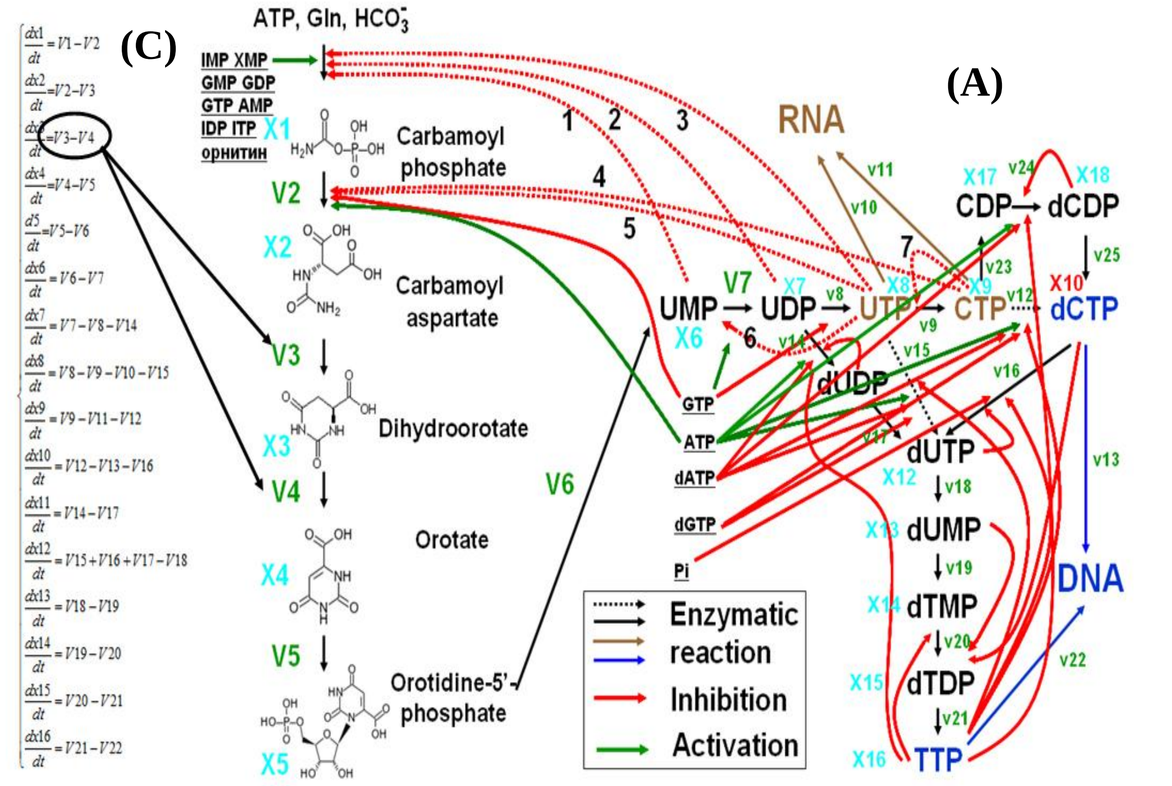

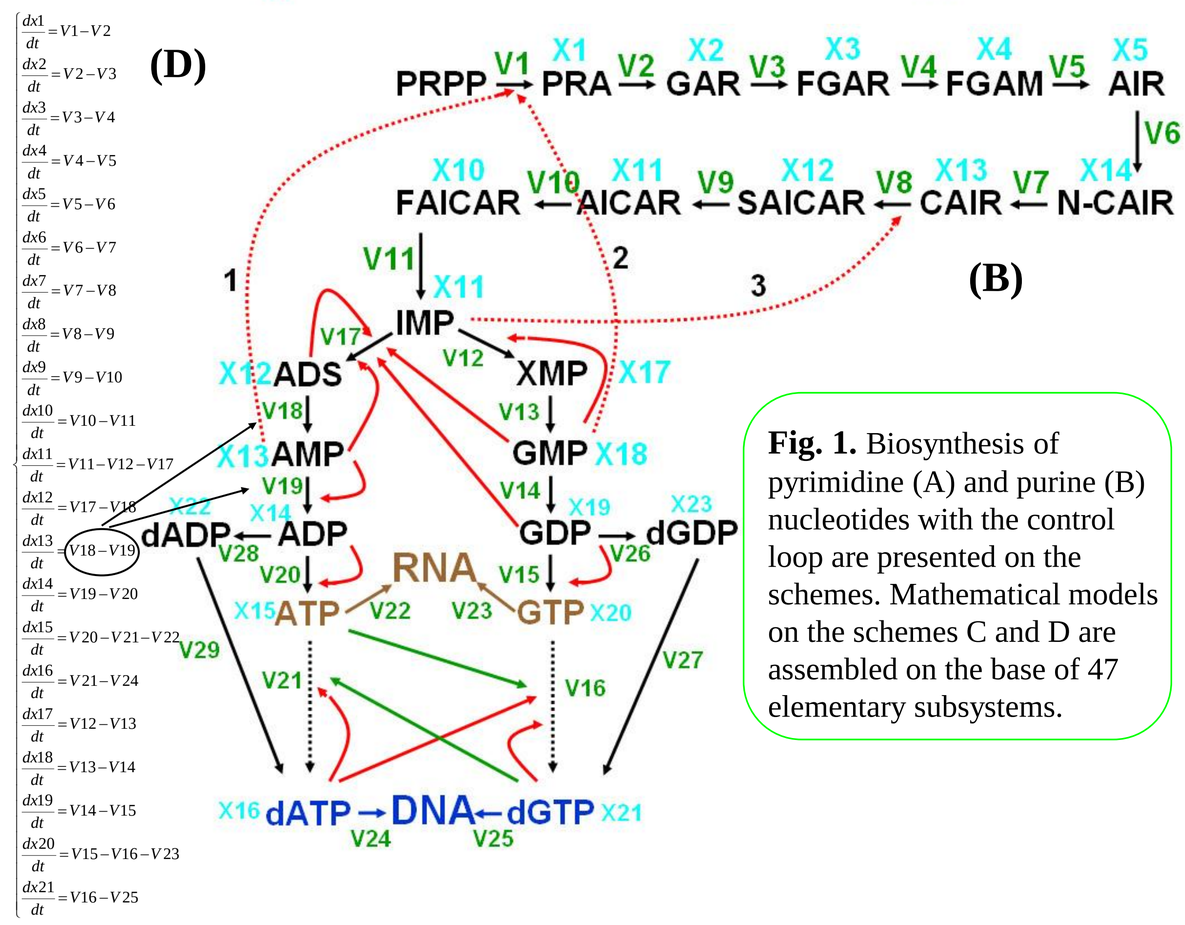

Fig. 1. Biosynthesis of pyrimidine (A) and purine (B) nucleotides with the control loop are presented on the schemes. Mathematical models on the schemes C and D are assembled on the base of 47 elementary subsystems.

For investigation of solution oscillations from model MPir the sub-model (M), containing 7 regulatory loops controlling CTP biosynthesis from carbamoyl phosphate, was allocated. Values of parameters of the (M) model are presented in Tab.1.

$$(M)\begin{cases}\frac{dX1}{dt}=V1-V2\\ \frac{dX2}{dt}=V2-V3\\ \frac{dX3}{dt}=V3-V4\\ \frac{dX4}{dt}=V4-V5\\ \frac{dX5}{dt}=V5-V6\\ \frac{dX6}{dt}=V6-V7\\ \frac{dX7}{dt}=V7-V8\\ \frac{dX8}{dt}=V8-V9-V10\\ \frac{dX9}{dt}=V9-V11\end{cases}$$

where

$$V1=\frac{k1}{1+\left(\frac{x6}{kump1}\right)^{hump1}+w1*\left(\frac{x7}{kudp1}\right)^{hudp1}+w1*\left(\frac{x8}{kutp1}\right)^{hutp1}},$$

$$V2=\frac{k2*(x1)^{hcap}}{(Kmcap)^{hcap}+(x1)^{hcap}}*\frac{1+w2*\frac{x9}{kctp21}}{1+w2*\left(\frac{x8}{kutp2}\right)^{hutp21}+w2*\frac{x9}{kctp22}+w2*\left(\frac{x8}{kutpctp}\right)^{hutp22}},$$

$$V3=\frac{k3*x2}{Kmcaasp+x2},V4=\frac{k4*x3}{Kmdoroa+x3},V5=\frac{k5*x4}{Kmoroa+x4},V6=\frac{k6*x5}{Kmomp+x5},$$

$$V7=\frac{k7*x6}{kmump+x6}*\frac{1}{1+w1*\left(\frac{x8}{kutp7}\right)^{hutp7}},V8=\frac{k8*x7}{Kmudp+x7},$$

$$V9=\frac{k9*(x8)^{hutp9}}{(x8)^{hutp9}+\left(1+w1*\frac{x9}{kctp9}\right)*(Kmutp9)^{hutp9}},V10=k10*x8,V11=k11*x9$$

Tab. 1. Parametrs of model (M)

k1	30.33 [mM/sec]	**k10**	0.38 [1/sec]	**kctp22**	0.06 [mM]	**hutp7**	2.4
k2	15.15 [mM/sec]	**k11**	0.59 [1/sec]	**kutpctp**	0.6 [mM]	**Kmudp**	0.09 [mM]
k3	1.05 [mM/sec]	**kump1**	0.04[mM]	**hutp22**	3.2	**hutp9**	1.4
k4	0.65 [mM/sec]	**hump1**	1.4	**Kmcaasp**	0.47 [mM]	**kctp9**	1.1 [mM]
k5	0.59 [mM/sec]	**hcap**	2.2	**Kmdoroa**	0.0275 [mM]	**Kmutp9**	0.3 [mM]
k6	1.45 [mM/sec]	**Kmcap**	0.2 [mM]	**Kmoroa**	0.04[mM]	**kudp1**	0.015 [mM]
k7	1.2 [mM/sec]	**kctp21**	0.27 [mM]	**Kmomp**	0.006 [mM]	**hudp1**	1.4
k8	2.52 [mM/sec]	**kutp2**	9 [mM]	**Kmump**	0.05 [mM]	**kutp1**	0.64 [mM]
k9	0.35 [mM/sec]	**hutp21**	2	**kutp7**	0.54 [mM]	**hutp1**	1.4
w1	1	**w2**	1				

Model M≡M(1,2,3,4,5,6,7) are contained 7 control loops, which negative control enzyme reaction velocity – V1, V2, V7, V9. For model reception M(1,4,5) and M(1) it is necessary to exclude number control loop. In the model M (1,4,5) w1=0,w2=1. In the model M(1) w1=w2=0.

However, earlier in [*Rodriguez et.al_2005*], the model which contains control loop 1,4,5 has been described, but for it fluctuations have not been revealed. As has shown the comparative analysis in the model of M(1,4,5) containing the same contours 1,4,5, value of factor Hill hump1, estimated by us on the basis of the data from [*Robin_1989*], it is equal 1.4, and this factor is equal in model Rodriguez 1. Replacement of value of parameter hump1 in M(1,4,5) on 1 also led to an establishment of a stationary mode of functioning. Nevertheless in model M(1) containing only one contour 1, change of value of factor Hill hump1 on 1 did not lead to disappearance of an oscillatory mode. The made numerical experiments confirm key value of a contour 1 for formation of an oscillatory mode. Introduction in system of additional mechanisms limits area of existence of oscillatory modes to values of parametre Hill above 1.