GENE NETWORK RECONSTRUCTION AND MATHEMATICAL MODELING OF *E. COLI* RESPIRATION: REGULATION OF F0F1-ATP SYNTHASE BY METAL IONS

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SUMMARY

Motivation: Development of an *in silico* cell as a computer resource for simulation and analysis of processes within living cells is an urgent task of systems biology and computational biology. Within this direction, it is necessary to develop mathematical models of the genetic regulation of cell metabolic pathways, in particular, the regulation of *E. coli* respiration enzymes.

Results: By using the GeneNet technology, we reproduced the gene network of the regulation of respiration in the *E. coli* cell. Mathematical models were constructed by the method of generalized Hill functions. The models describe the velocities of enzymatic reactions.

Availability: The diagram of the gene network "Respiration" is available through the GeneNet viewer at http://wwwmgs.bionet.nsc.ru/mgs/gnw/genenet/viewer/index.shtml.

INTRODUCTION

Fine mechanisms of expression regulation of the respiration enzymes in *E. coli* allow to cells well living in various conditions. More than 20 various enzymes provide this diversity forming respiration chain, which consist of pairs of interacting enzymes: oxidizer-deoxidizer. Optimal pair (or set of pairs) choice depends on a lot of parameters, but the significative ones are the oxygen concentration and presence of some substrate in the environment. Complex analysis of such complicated processes as respiration is very difficult without computer modeling. Stating the problem of *in silico* cell model development we have used the gene network reconstruction methodology and modeling in the terms of elementary processes. On this evidence the gene network of respiration in *E. coli* was reconstructed and the enzymatic processes database was developed.

METHODS AND ALGORITHMS

The gene network of regulation respiration was reconstructed with the GeneNet technology (Ananko *et al.*, 2005). Mathematical models were constructed by the method of generalized Hill functions (Likhoshvai, Ratushny, 2006).

RESULTS AND DISCUSSION

The GeneNet technology (Ananko *et al.*, 2005) was applied to reconstruction of the gene network regulating the respiration of *E. coli* cells. The gene network of respiration contains description of genetic regulation of operons, controlling enzymes synthesis of the respiration chain and metabolic processes, providing respiration of *E. coli* cells. The fragment of this network is shown on the Fig. 1. Section "Respiration" of the GeneNet contains description of 22 operons, 27 mRNA, 111 proteins, 64 different metabolites and others small molecules, and 313 interrelations between components. The information has been extracted from 241 scientific papers.

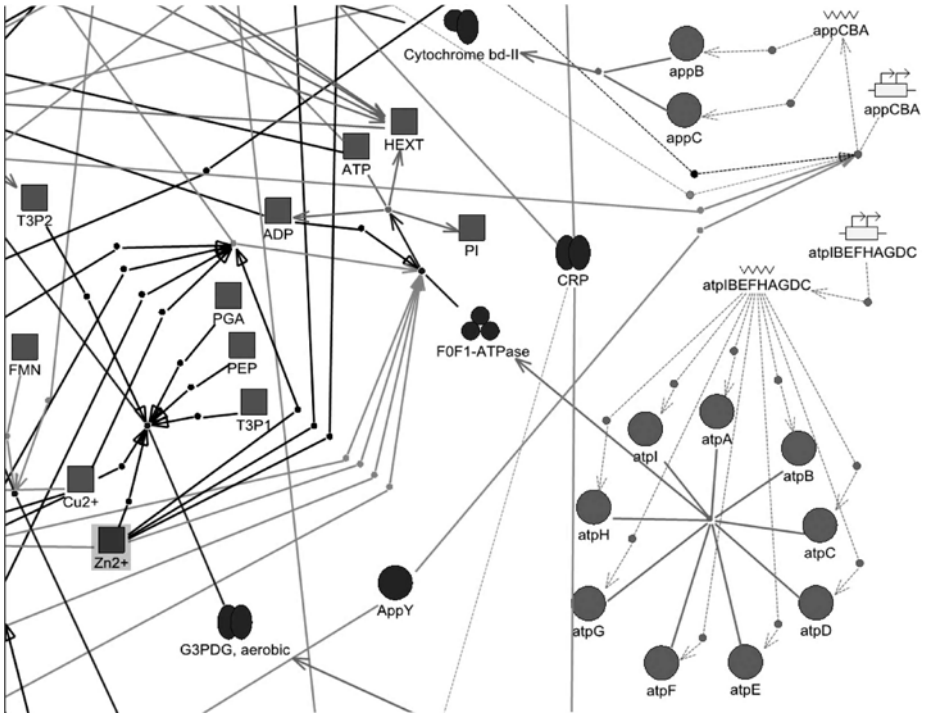

Figure 1. Fragment of the "Respiration" GeneNet diagram.

We also developed a database storing experimental data on the dynamic behaviour of the gene network components. Its content is illustrated in Table 1. As seen, at the present day, the database accumulates descriptions of ~340 constants and dynamic profiles involved in kinetics of the processes taking place in gene network of respiration.

Table 1. The parameters of enzymatic reactions, providing respiration of *E. coli* cells (the data K_m include the values of both K_m of substrates and cofactors)

Class	Dynamic	Enzymatic reaction parameters						
	Profile	Michaelis constant K_m	Constant of catalytic activity k_{cat}	Maximal velocity V_{max}	Dissociation constant K_d	Hill coefficient n^H	Constant of activation K_{act}	Constant of inhibition K_i
Amount	92	122	22	47	16	11	2	26

Mathematical models of 19 enzymatic reactions were constructed. Parameters of the models were determined by numerical experiments. The results of calculation of steady-state and dynamic parameters deduced from the models were in agreement with experimental data. Now, we consider regulation of the reaction catalyzed by the membrane-

bound F_0F_1-ATPase, coded by the *atpABCDEFGHI* operon, as an example of simulation of molecular processes in the gene network of in *E. coli* (Fig. 1). This enzyme catalyzes the reaction of ATP synthesis and hydrolysis in accordance of the following equation.

$ATP \leftrightarrow ADP + P_i + 4H^+$

Moreover the ATP synthesis carries in aerobic conditions and is provided of those respiration enzymes, which are capable of forming the proton gradient in the chain of electrons transport. ATP hydrolysis carries in anaerobic conditions and enzyme's catalytic domain (F_1) contains three binding sites of ATP whose affinity depends on the Mg^{2+} presence (Weber *et al.*, 1996). It determines the complex cooperative effect of ATP and Mg^{2+} on the ATPase activity. In the proposed model we also take into account ATP and ADP effects of substrate and competetive inhibition respectively. There is the activator effect of Na^+ on the ATPase activity at the concentrations lower than 15–20 mM and inhibitor effect at the higher concentrations and Na^+ influence on the inhibitory effect of K^+ (Koebmann *et al.*, 2002). Without going into details of the enzymatic activity mechanism which as is evident from the above description, is very complex, note that the method of generalized Hill functions allows for obtaining a very compact description of the overall processes considered. Taking into account effects of Mg^{2+}, Na^+, and K^+ described above on the enzymatic activity, the rate of reaction, catalyzing by the ATPase enzyme may be represented in a generalized form by the following equation:

$$V = V_{max} \cdot \frac{ATP / K_{m,ATP}}{1 + \frac{ATP}{K_{m,ATP}} + \frac{ADP}{K_{i,ADP}}} \cdot \frac{1 + \left(\frac{ATP}{k_{i,ATP}}\right)^{h_{i,ATP}}}{1 + \left(l_{i,ATP} \cdot \frac{ATP}{k_{i,ATP}}\right)^{h_{i,ATP}}} \cdot f_{Mg} \cdot f_{Na} \cdot f_K, \quad (1)$$

where $K_{m,ATP}$ – Michaelis constant for ATP, $K_{i,ADP}$ – inhibitor constant for ADP, $k_{i,ATP}$, $l_{i,ATP}$ – constants describing efficiency of ATP substrate inhibition, $h_{i,ATP}$ – constant of substrate inhibition nonlinearity. The subformulas f_{Mg}, f_{Na}, f_K, describing effects of magnesium, sodium and potassium respectively on the enzyme activity are the generalized Hill functions:

$$f_{Mg} = \frac{\frac{k_{0Mg}}{1 + \left(ATP / k_{i,Mg0ATP}\right)} + \frac{l_{aMg}(ATP) \cdot Mg}{k_{aMg}(ATP)}}{1 + \frac{Mg}{k_{aMg}(ATP)} \cdot \left(1 + \left(\frac{Mg}{k_{iMg}(ATP)}\right)^{n_{iMg}(ATP)}\right)},$$

where $k_{0,Mg}$, $k_{i,Mg0ATP}$ – describing ATP effect at the low magnesium concentrations, the subformulas $k_{a,Mg}$, $k_{i,Mg}$, $l_{i,Mg}$ and $n_{i,Mg}$, describing the efficiency and nonlinearity of magnesium influence depending on ATP concentration are also generalized Hill functions (not presented).

$$f_{Na} = \frac{1 + \left(\frac{l_{aNa} \cdot Na}{k_{aNa}}\right)^{n_{aNa}} \cdot \left(1 + \left(\frac{Na}{k_{iNa}}\right)^{n_{iNa}}\right)}{1 + \left(\frac{Na}{k_{aNa}}\right)^{n_{aNa}} \cdot \left(1 + \left(\frac{l_{iNa} \cdot Na}{k_{iNa}}\right)^{n_{iNa}}\right)},$$

where k_{aNa}, l_{aNa}, n_{aNa} – constants of efficiency and nonlinearity of sodium activator effects at the low concentrations, $k_{i,Na}$, $l_{i,Na}$, $n_{i,Na}$ – constants of efficiency and nonlinearity of sodium inhibitor effects at the higher concentrations.

$$f_K = \frac{1 + \left(\frac{K}{k_{iK}(Na)} \right)^{n_{iK}}}{1 + \left(\frac{l_{iK}(Na) \cdot K}{k_{iK}(Na)} \right)^{n_{iK}}},$$

where subformulas k_{iK} and l_{iK} are the generalized Hill functions (not presented) and describe the efficiency of potassium influence depending on sodium concentration and $n_{i,Mg}$ – constant of nonlinearity of potassium concentration.

Note that characteristic of the mathematical model proposed, constructed using a generalized Hill function, is a considerable simplicity (compared with the molecular biological processes simulated); nonetheless, it provides a very good fit of the experimental data and numerical calculations.

Fig. 2 shows the results of calculations using model (1) and their comparison with the experimental data on the effects of various Mg^{2+}, Na^+, and K^+ and ATP concentrations on the ATPase enzymatic activity. This comparison demonstrates a high adequacy of the model of genetic expression regulation of the gene considered.

The example given above demonstrates a strategy of metabolic reactions modeling, which is also used for modeling and description of complex molecular-genetic processes (Likhoshvai *et al.*, 2006, this issue). In general, the work shows the reconstruction of fragment being the component of complex gene network of respiration regulation in *E.coli* cell. It is the first and essential step in constructing of complete kynetic model of any molecular-genetic system. It is nesessary to say that the proposed methodology of constructing the elementary kynetic models allows us to reduce the model dimension keeping the complex effects and description adequacy. The models of the all enzymatic reactions of gene network "Respiration", including the model described above will be an inextricable part of the "*in silico* cell" computer resource.

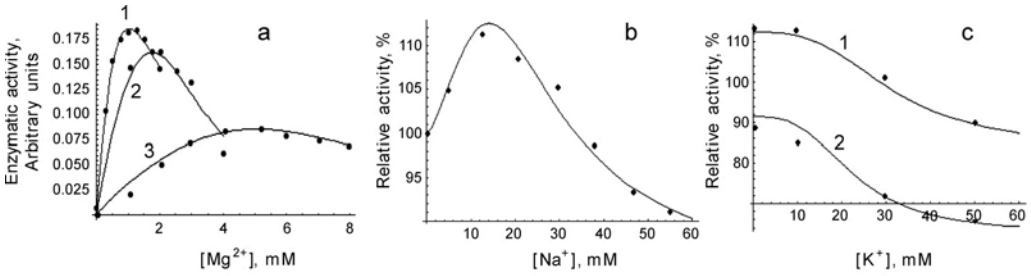

Figure 2. The effects of *a* – Mg^{2+} concentration at various ATP concentrations, *b* – Na^+ concentration, *c* – K^+ concentration at various Na^+ concentrations on the ATPase enzymatic activity. The enzyme activity was measured (a) with the ATP concentration of 2.5 mM, 5 mM and 10 mM (1,2,3 respectively); (b) ATP (2.5 mM), Mg^{2+} (1 mM); (c) ATP (2.5 mM), Mg^{2+} (1 mM), Na (1, 15 mM, 2, 55 mM). (a) Dots indicate experimental data from (Koebmann *et al.*, 2002), and curves are the results of simulation according to model (1) with the estimated parameters.

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