AROMATIC AMINO ACID BIOSYNTHESIS IN *ESCHERICHIA COLI*: GENERALIZED HILL FUNCTION MODEL OF THE TRYPTOPHAN-SENSITIVE 3-DEOXY-D-ARABINO-HEPTULOSONATE-7-PHOSPHATE SYNTHASE REACTION DEMONSTRATE COMPLICATED MECHANISM

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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.

Results: By using the GeneNet technology, we reproduced the gene network of the regulation of aromatic amino acid biosynthesis in the *E. coli* cell. Mathematical models were constructed by the method of generalized Hill functions. The models describe the efficiency of enzymatic systems and regulation of expression of related genes. Mathematical model of the enzyme tryptophan-sensitive 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase reaction demonstrate complicated mechanism.

Availability: Models are available on request. The diagram of the gene network regulating aromatic amino acid biosynthesis in *E. coli* is available through the GeneNet viewer at http://wwwmgs.bionet.nsc.ru/mgs/gnw/genenet/viewer/index.shtml.

INTRODUCTION

The pathway of aromatic amino acid biosynthesis shows the common pathway (shikimate pathway) and three terminal pathways in which chorismate is converted to phenylalanine, tyrosine, and tryptophan, respectively (Pittard, 1996). In *E. coli*, these pathways involve 23 enzymes coded by the genes *aroF*, *aroG*, *aroH*, *aroB*, *aroD*, *aroE*, *aroK*, *aroL*, *aroA*, *aroC*, *tyrA*, *tyrB*, *aspC*, *pheA*, *trpE*, *trpD*, *trpC*, *trpA*, and *trpB*. Many of these genes, as well as the activity of the corresponding enzymes are controlled by end products, namely, tyrosine, phenylalanine, and tryptophan.

We reconstructed the gene network regulating aromatic amino acid biosynthesis in *E. coli* and developed mathematical models describing the operation of some individual enzymatic systems[3].

[3] The abbreviations used are: 3DDAH7P, 3-deoxy-D-arabino-heptulosonate-7-phosphate; DAHPS, 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase; Trp, L-tryptophan.

METHODS AND ALGORITHMS

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

RESULTS

The GeneNet technology (Ananko *et al.*, 2005: Khlebodarova *et al.*, 2006) was applied to reconstruction of the gene network regulating aromatic amino acid biosynthesis in *E. coli* (Fig. 1). Section "Aromatic Amino Acids" of the GeneNet contains description of 30 proteins, 11 operons, 4 genes, 45 small molecules, and 130 interrelations between components. The information has been extracted from 256 scientific papers.

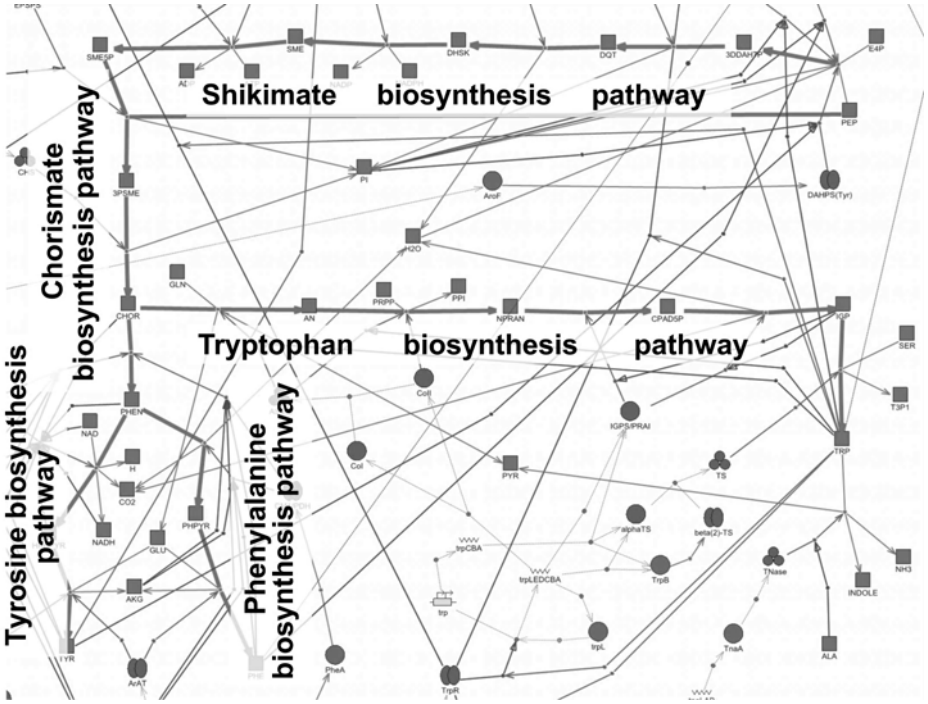

Figure 1. Fragment of the "Aromatic Amino Acids" GeneNet diagram.
Bold arrows indicate main pathways.

The gene network of aromatic amino acid biosynthesis contains description of five biosynthetic pathways, namely, Shikimate biosynthesis, Chorismate biosynthesis, Tyrosine biosynthesis, Phenylalanine biosynthesis, and Tryptophan biosynthesis, including 6, 4, 4, 3, and 6 enzymatic reactions, respectively.

We also developed a database storing experimental data on the dynamic behaviour of the gene network components (Khlebodarova *et al.*, 2006). Mathematical models of all 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 enzyme tryptophan-sensitive 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHPS(Trp)), coded by the *aroH* gene, as an example of simulation of molecular processes in the gene network of aromatic amino acid biosynthesis in *E. coli.* This enzyme is one of three differentially

regulated isozymes that catalyze the first step of aromatic biosynthesis, the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) to form 3-deoxy-D-arabino-heptulosonate-7-phosphate (3DDAH7P). Here is the reaction of synthesis of 3DDAH7P from E4P and PEP, catalyzed by DAHPS: E4P + PEP + H2O $\rightarrow$ 3DDAH7P.

The enzyme is homodimeric and has two independent inhibitor binding sites. DAHPS(Trp) displays sigmoid kinetics with respect to both substrates, E4P and PEP. Both catalytic activity and substrate affinity of the DAHPS(Trp) are dependent on the species of activating metal ion. L-Tryptophan (Trp) binding decreases k_{cat}, decreases positive homotropic cooperativity for both substrates and activates the enzyme at low concentrations of E4P (Akowski, Bauerle, 1997).

The enzymatic reaction has a very intricate mechanism of DAHPS(Trp) regulation by Trp. With regard to the effect of Trp on various parameters of the molecular system under consideration, the rate of the enzymatic reaction can be expressed as:

$$V = \frac{k_{cat} \cdot e_0 \cdot \left(S_1 / \left(K_{m,S_1} \cdot f_2\right)\right)^{h_{S_1} \cdot f_3} \cdot \left(S_2 / K_{m,S_2}\right)^{h_{S_2} \cdot f_4}}{P_2 / K_{i,P_2} + \left[1 + \left(S_1 / \left(K_{m,S_1} \cdot f_2\right)\right)^{h_{S_1} \cdot f_3}\right] \cdot \left[1 + \left(S_2 / K_{m,S_2}\right)^{h_{S_2} \cdot f_4}\right]} \cdot f_1,$$

$$f_1 = \frac{1}{1 + kl_{R,V_{max}} \cdot \frac{R}{k_{R,V_{max}} + R}}, \quad f_2 = \frac{1}{1 + kl_{R,K_{m,S_1}} \cdot \frac{R^{h_{R,K_{m,S_1}}}}{k_{R,K_{m,S_1}}^{h_{R,K_{m,S_1}}} + R^{h_{R,K_{m,S_1}}}}}, \tag{1}$$

$$f_3 = \frac{1}{1 + kl_{R,h_{S_1}} \cdot \frac{R^{h_{R,h_{S_1}}}}{k_{R,h_{S_1}}^{h_{R,h_{S_1}}} + R^{h_{R,h_{S_1}}}}}, \quad f_4 = \frac{1}{1 + kl_{R,h_{S_2}} \cdot \frac{R^{h_{R,h_{S_2}}}}{k_{R,h_{S_2}}^{h_{R,h_{S_2}}} + R^{h_{R,h_{S_2}}}}},$$

where V is the rate of the reaction; e_0 is the concentration of the enzyme DAHPS(Trp); S_1, S_2, P_1, P_2, and R are the concentrations of E4P, PEP, PI, 3DDAH7P, and Trp, respectively; k_f is the catalytic constant; $K_{m,S1}$ and $K_{m,S2}$ are the Michaelis–Menten constants for the substrates E4P and PEP, respectively; $K_{i,P2}$ is the constant of inhibition by the 3DDAH7P product; h_{S1} and h_{S2} are constants determining the nonlinearity of the effect of the substrates E4P and PEP on the reaction rate, respectively; $kl_{R,Vmax}$ is the constant determining the maximum degree of reaction rate inhibition by Trp; $k_{R,Vmax}$ is the constant of efficiency of the effect of Trp on the maximum rate of the reaction; $kl_{R,k}$ is the constant determining the maximum effect of Trp on the constant designated by k, where k assumes a character value from the set $\{K_{m,S1}, h_{S1}, h_{S2}\}$; $k_{R,k}$ is the constant determining the efficiency of the effect of Trp on constant k; and $h_{R,k}$ is the constant determining the nonlinearity of the effect of Trp on constant k.

Although simple, the model provides good agreement between experimental data and simulation results. Fig. 2*a*, *b* present the results of calculations according to model (1) compared with experimental data on the effect of various Trp concentrations on the rate of the reaction catalyzed by the enzyme DAHPS(Trp). Apparently, the proposed model of the genetic regulation of the gene under study is fairly precise.

DISCUSSION

Reconstruction of the gene network and development of mathematical models describing the efficiency of operation of enzymatic systems and regulation of genes coding for these enzymes must be the initial stage of the development of an overall

kinetic model of the gene network of aromatic amino acid biosynthesis. The advantage of the method suggested is that it enables to construct appropriate mathematical models with the minimal complexity of description of the modelled processes in conditions of the lack of knowledge about fine mechanisms of reactional process. It was demonstrated by the example of the model describing regulation of DAHPS(Trp) enzyme activity by Trp. The model will be an inextricable part of the "*in silico* cell" computer resource.

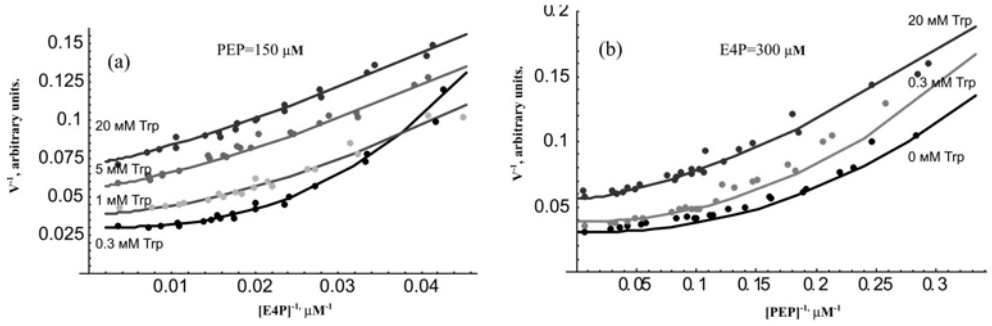

Figure 2. Effect of various Trp concentrations on the rate of the enzymatic reaction catalyzed by the DAHPS(Trp). Experimental conditions: *а* – PEP = 150 mM, *б* – E4P = 300 mM. On the X axis the reverse concentrations of substrates are plotted; on the Y axis, the reverse rates on the enzymatic reactions. Dots indicate experimental data according to (Akowski, Bauerle, 1997); curves are the results of simulation according to model (1); parameter values: k_f = 20.6 s-1; $K_{m,S1}$ = 35 mM; $K_{m,S2}$ = 5.3 mM; h_{S1} = 2.6; h_{S2} = 2.2; $K_{i,P1}$ = 1 mM; $kl_{R,Vmax}$ = 1.7; $k_{R,Vmax}$ = 5 mM; $kl_{R,KmS1}$ = 0.85; $k_{R,KmS1}$ = 25 mM; $h_{R,KmS1}$ = 0.6; $kl_{R,hS1}$ = 1.1; $k_{R,hS1}$ = 1 mM; $h_{R,hS1}$ = 1; $kl_{R,hS2}$ = 0.47; $k_{R,hS2}$ = 1 mM; $h_{R,hS2}$ = 2.

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REFERENCES

Akowski J.P., Bauerle R. (1997) Steady-state kinetics and inhibitor binding of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (tryptophan sensitive) from *Escherichia coli*. *Biochemistry*, **36**, 15817–15822.

Ananko E.A. *et al.* (2005) GeneNet in 2005. *Nucl. Acids Res.*, **33**, D425–D427.

Khlebodarova T.M. *et al.* (2006) *In silico* cell II. Information sources for modeling *Escherichia coli* metabolic pathways. *This issue*.

Likhoshvai V.A., Ratushny A.V. (2006) *In silico* cell I. Hierarchical approach and generalized hill functions in modeling enzymatic reactions and gene expression regulation. *This issue.*

Pittard J.A. (1996) Biosynthesis of the aromatic amino acids. In Neidhardt F.C. (ed.), *Escherichia coli and Salmonella.* In *Cellular and Molecular Biology*. Second Edition. Washington, DC: American Society for Microbiology Press., **1**, 458–484.