Integration of genetic and enzymatic controls in metabolic pathways

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The bacterial metabolic machinery and its regulation are a complex system and involve many components: metabolites and enzymes. The reconstruction of the regulatory networks of *B. subtilis including the enzymatic reactions, the control of enzymatic activities and the genetic regulations (see [1]) allowed us to identify the main regulation structures of metabolic pathways. We noticed a strong interplay between the metabolic pathways and the genetic regulations. Then, in order to break the intrinsic complexity of such huge system and to reveal somewhat an order, we propose a new suitable notion of subsystems (modules) using mathematical dynamic models associated to these structures.*

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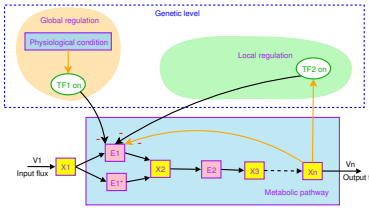
End-product control structure



Dynamical system

$$\begin{cases} \dot{x}_1 = \nu_1 - E_1 f_1(x_1, x_n, p) - E_1^* f_1^*(x_1) \\ \dot{x}_2 = E_1 f_1(x_1, x_n) + E_1^* f_1^*(x_1) - E_2 f_2(x_2, p) \\ \vdots \\ \dot{x}_n = E_{n-1} f_{n-1}(x_{n-1}, p) - E_n f_n(x_n) \\ \dot{E}_1 = g(x_n, q) - \mu E_1 \end{cases}$$

x_i : the concentration of the metabolite X_i,
E_i : the concentration of the enzyme E_i
ν_1 : the input flux (the flux supply)
$E_n f_n(x_n)$: represents the output flux (the flux demand)
f_i : the reaction rate of the enzyme E_i
g : the genetic control of E_1
p : a vector of external factors (cofactors)
q : a vector of global regulators
μ : the growth rate. (i.e $\dot{V}(t) = \mu V(t)$)

The study of the equilibrium regime in exponential phase allows us to deduce some biological properties, and the ability to breakdown the huge metabolic network on subsystems(modules).

Equilibrium regime

$$\begin{cases} \bar{E}_1 = \dfrac{g(\bar{x}_n)}{\mu}, \\ f_1(\bar{x}_1, \bar{x}_n) g(\bar{x}_n) = \mu E_n f_n(\bar{x}_n), \\ \nu_1 = E_n f_n(\bar{x}_n) \end{cases}$$

and for any $i = \{2, \ldots, n-1\}$, $\bar{x}_i = f_i^{-1}\left(\dfrac{E_n f_n(\bar{x}_n)}{E_i}\right)$.

Saturation condition: $E_i M_i = E_i \lim\limits_{x_i \to +\infty} f_i(x_i) > E_n f_n(\bar{x}_n)$.

Biological properties of the equilibrium regime

The prediction of the equilibrium behavior of such linear metabolic pathway in the exponential phase can be simple even though it is composed of many reactions

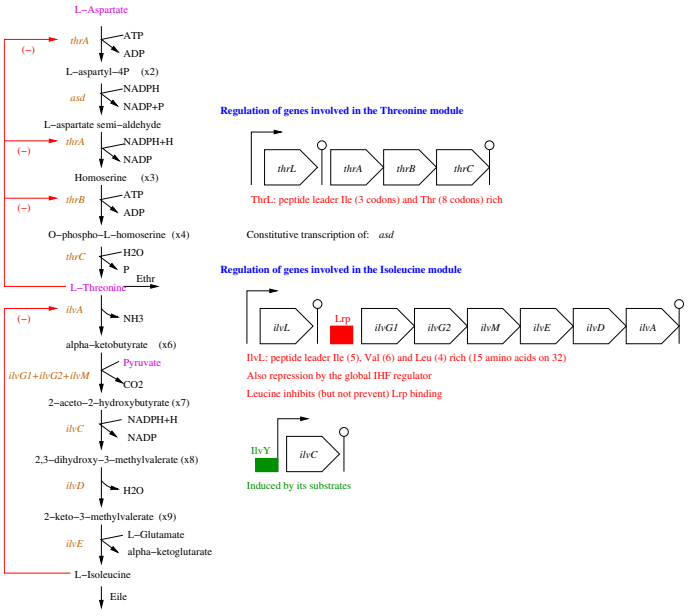
- The end product depends only on the genetic and allosteric controls of the first enzyme E_1.
- The nature of the intermediate enzymes $E_2, \ldots, E_{n-1}$ has no impact on the equilibrium regime of the end product concentration and only impact the equilibrium of the intermediate metabolite concentrations.
- The way to increase the output flux is to decrease the end product concentration $\bar{x}_n$.
- The equilibrium of the intermediate metabolites are increasing when the output flux are increasing.
- The presence of the **isoenzyme** can allow the metabolic pathway to have a larger flux increasing the concentration of the isoenzyme.
- **Cofactors** act on (i) the first enzyme E1 (ii) the last enzyme En (iii) the intermediate enzymes.
  (i) and (ii)⇒ a direct impact on the equilibrium of the end product, (iii)⇒ no impact on the equilibrium only if it leads to saturate an intermediate step.
- **Global regulation** is able to modify the enzymes production of many pathways (pleiotropic factors (CcpA,TnrA)[1]) ⇒ modifies the relation between the end product concentration, the flux demand and the enzyme concentration. For the operon case, it modifies all the concentrations of the pathway.

Although this study concerns the particular end product structure, the obtained results could be extended to other control structures [2].

How to break down the huge metabolic network into modules

To reveal an order in such a huge system, we introduce a suitable notion of subsystems (modules).

- The key role of irreversible steps,
- The key role of the regulated enzymes and the metabolite associated to their regulations,
- The coordination between different modules: compatibility of the fluxes through key metabolites, coordination with a global regulator.



References

[1] A. Goelzer et al.: Reconstruction and analysis of the genetic and metabolic regulatory networks of the central metabolism of Bacillus subtilis. BMC systems Biology, 2008.

[2] F. Bekkal-Brikci, A. Goelzer, V. Fromion: Integrated enzyme controls in metabolic network analysis. In preparation.

[3] F. C. Neidhardt: Escherichia coli and Salmonella: cellular and molecular biology, 2nd edition, Chapter 32. American Society of Microbiology, 1996.

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