A MODEL OF TRYPTOPHAN BIOSYNTHESIS REGULATION

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Summary

Motivation: Modeling of metabolite biosynthesis in bacterial culture and, particularly, constructing a model for gene expression regulation present a long-standing challenge (see Elf *et al*., 2001; Santillam, Mackey, 2001 for details). Modeling approaches based on differential equation tools or stochastic processes are associated with known computational limitations as well as difficulties in interpreting the result. The latter arise due to indirectness in describing biological processes with such mathematicaltools. Therefore, development of more direct and computationally clear modeling techniques is necessary.

Results: We propose a methodological approach based on the generalized automata theory (directed graphs with potentially infinite number of marks). See the Discussion section too.

Introduction

Let us define the multitude of possible states as a set of vertices of the directed graph with each edge denoting transition between possible states. Each vertex is assigned a rational number (from 0 to 100) corresponding to the percentage of bacteria in culture with the fixed operon (or, more precisely, the orthologous set of operons) being in the state ascribed to this vertex. Phase dynamics on this graph is the change in value distribution of this percentage and average characteristics of the culture.

Let us illustrate the approach with tryptophan synthesis as an example. We consider dynamics of the tryptophan synthesis pathway enzyme concentrations, the metabolite (tryptophan) concentration and the active repressor concentration. To simplify, we consider one enzyme and one repressor, although the model can easily be extended to incorporate several enzymes and repressors/activators. Obviously, metabolite concentrations differ in various bacterial cells at any given instance. Therefore, the model operates with average values (similarly to Elf *et al*., 2001; Santillam, Mackey, 2001 and other studies) assuming that the average and real concentrations correlate similarly. Concentration heterogeneity can be incorporated into the model by specifying the concentration of each substance at each operon state. The probability of RNA-polymerase binding (at each time phase) is a function of the active repressor concentration; similarly, other probabilities are functions of relevant concentrations. The increase in tryptophan concentration entails (i) the increase in probability of the ribosome shifting from the regulatory sequence, (ii) stronger inhibition of the enzyme by tryptophan, (iii) depletion of the amount of tryptophan synthesized by 1 % enzyme, (iv) buildup of the active repressor. These relationships were expressed so as to incorporate known biological properties and general trends of the biosynthesis. The model also takes into account processes of tryptophan metabolism, decay and transport from the environment, as well as growth of the bacterial culture.

Methods and Algorithms

Our model of tryptophan biosynthesis incorporates (1) a multitude of conditional states of bacteria and the function (perhaps, probabilistic), which, given percentage distribution of bacterial states

and current concentrations of the enzyme, tryptophan and active repressor, predicts a new distribution and new substance concentrations for the next phase.

We introduce the terminal state interpreted as "end of game", which is not assigned the parameter percentage distribution. The amount of bacteria falling into the terminal state is immediately compensated by the income of bacteria at the initial state ("the operon unattended"). Thus, a new percentage distribution is uniquely defined by the old distribution with a set of functions f_q, where for each state q the function $f_q(q')$ determines the percentage distribution of q'-bacteria after transition from state q at the end of the phase. For the sake of simplicity, we let the synthesis success or failure be determined by the succession, in which the ribosome and RNA-polymerase arrive at their corresponding "finishing points". For ribosome, it is located at a given distance from the regulatory codon area (we assume that the regulatory codons are adjacent; hereafter the regulatory codon is referred to as a codon), and for RNA-polymerase – at a given distance from the pause site. The special case when the ribosome does not bind before the polymerase has reached the "finishing point" we consider a failure. We consider different modifications of function f_q and the function defining concentrations of the enzyme, repressor and tryptophan at a successive phase by those at the preceding phase.

Implementation and Results

We assumed that (1) the procession of RNA-polymerase and ribosome are equal and constant, and (2) the pause time is constant. The time scale (phases) was defined as the time required for processing of one nucleotide by RNA-polymerase. Under this assumption various parameters were taken from the review (Xie *et al*., 2003), private correspondences and roughly estimated from the empirical data. Varying numerical values of ambiguous parameters showed that they have little impact on modeled correlations (at least qualitatively). At the initial phase all bacteria were in state q_0 with zero concentrations of the enzyme, repressor and tryptophan. Over the first 135 phases when the enzyme was not yet produced it had zero concentration, that of tryptophan was close to zero and that of repressor fluctuated around 0.1. Over successive phases the enzyme concentration drastically increased reaching 0.66 in phase 139. In phase 153 the tryptophan content reached 1.68, which is close to its critical level of overexpression. Simultaneously, the repressor concentration also became high 1.75. Over the following phases, all concentrations began to diminish reaching 0.07 for the enzyme and tryptophan at phase 271, which corresponds to underexpression of tryptophan.

Such considerable fluctuations were observed for the initial phases, when the synthesis regulation was yet unstable. During upcoming phases they became smooth and ultimately disappeared. This process was continuous: after 1,500 phases concentrations of the enzyme, tryptophan and the repressor still varied nearly synchronously between 0.08 and 0.3, 0.08 and 0.14, 0.86 and 1.18, respectively. Such fluctuations can be accounted for biologically. Relevant experiments show that ultimate metabolite concentrations do not depend on their initial concentrations and distribution of the cell states in culture.

To compare the attenuator- and repressor-based regulatory strategies, two assays were conducted. In one of them, the contribution of repressor was reinforced (corresponding parameters were set to 0.1, which is two times less than standard values). As a result, fluctuation decay abruptly accelerated: after 1,500 phases they nearly disappeared with the concentration of enzyme stabilizing at 0.11 and that of tryptophan at 0.12. In the other assay, conversely, the repressor contribution to regulation was downgraded (corresponding parameters set to 0.5). This entailed longer and wider fluctuations: after 1,500 phases the concentration of the enzyme and tryptophan varied between 0.06 and 0.14, and between 0.06 and 0.17, respectively. Generally, this suggests that the repressor-mediated regulation is more efficient and responds quicker to expression of tryptophan than does the attenuator-based system. This result is in agreement with the generally accepted view that the repressor-mediated regulation is more sensitive.

In order to build a more realistic model, a certain degree of randomness was introduced in various ways. Thus, to allow for natural variations in cell demand for tryptophan, the corresponding parameter values were varied randomly around its average. This had little impact on modeled correlations, albeit the parameter fluctuations became more stochastic and did not disappear at the limit.

Discussion

The theory of directed graphs with potentially infinite number of marks can be used to generally describe transcription and translation processes and, particularly, processes of repressor- and attenuator-mediated regulation of operon expression and amino-acid biosynthesis. We propose to define a finite number of graph vertices corresponding to "states of the system". Transitions from one state to another are determined by factors like metabolite concentrations and values of certain random variables. For instance, the concentration of tryptophan mediates transition from the "pause-codon" state (when RNA-polymerase is in the last pause phase, and ribosome is at the last regulatory codon) to the "postpause-codon" state (when ribosome remains at the same codon) or to the "postpause-postcodon" state (when ribosome leaves the regulatory codon area). Besides, in each pause phase we dynamically assign graph vertices with numerical values corresponding to the proportion of bacteria in culture that are currently in a given phase along with some averaged characteristics of the culture. This technique was used to *in silico* model tryptophan biosynthesis regulation. The model confirmed, for instance, the empirical observation that the amino-acid concentration triggers a quicker response of the repressor-mediated rather than attenuator regulation system.

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