28 Hierarchical control of DNA supercoiling in *Escherichia coli*: how to study homeostatically controlled systems using control analysis

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Introduction

The cell is complex and adaptive. The complexity emerges in the nonlinear interactions of its many components. These interactions occur at the level of enzyme activities (e.g., through substrates, products or allosteric regulators), as well as gene expression (involving transcription, translation and degradation of RNA and protein). The latter confer a degree of plasticity to biological systems that is unprecedented by dead systems. Homeostatic control is just a weak example.

Some homeostatic control arises already in the kinetics of catalytic steps. The increase in product concentration will inhibit the stimulated step and activate the subsequent step in the pathway, poising the concentration of the product towards constancy, we refer to this as the metabolic part of homeostasis. Also the concentrations of the enzymes can be maintained homeostatically, i.e., through regulation of gene expression, which is the other part of homeostasis that we distinguish here.

Both types of homeostatic regulation are present in biological systems. However, most scientists focus on either the one ('metabolic control analysis') or the other ('molecular biology'). It might be of great interest to devise a method determining who is on the best track. Is metabolic regulation the determining step for

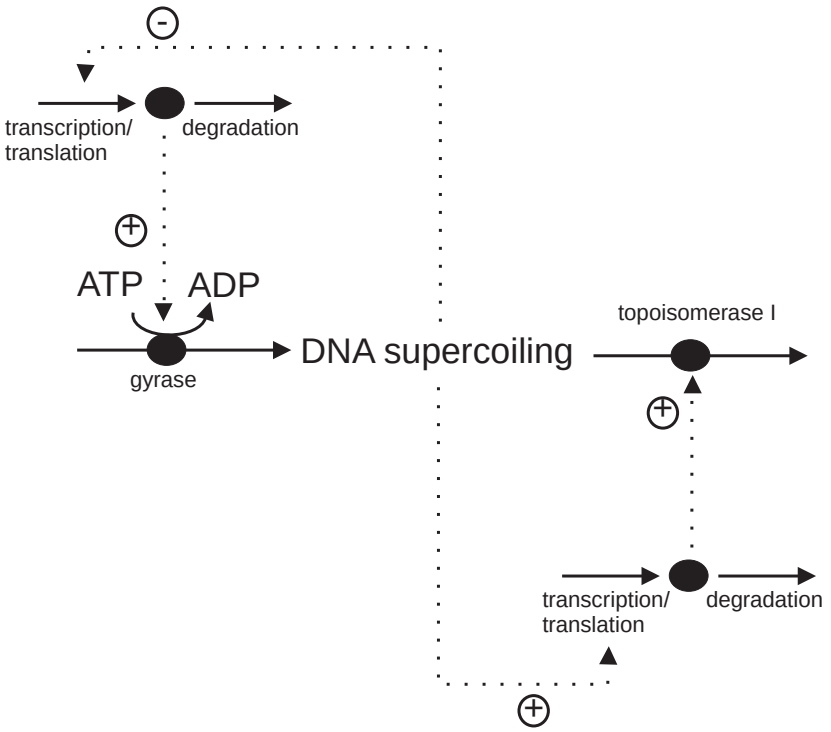

Fig. 28.1 Hierarchical control of DNA supercoiling. The metabolic level (control of DNA gyrase and topoisomerase I activities on supercoiling) and the gene expression level (transcription/translation and degradation of DNA gyrase and topoisomerase I) are indicated. The solid lines indicate flows and the regulatory influences are indicated by dotted arrows.

homeostasis, or is it gene expression that determines it all? We here demonstrate a new method (Snoep *et al.*, 2000) by which we determine quantitatively which of the two types of regulation is most important. In a familiar twist, we conclude that homeostasis in a living system may stem both from metabolic and from gene expression regulation and we show that for the particular case of DNA supercoiling in *E. coli*, paradoxically, the metabolic regulation seems to be the more important one.

Metabolic and hierarchical control analysis

Expressing control coefficients in elasticity coefficients, metabolic control analysis (MCA) links systemic properties to local characteristics. *M*CA is limited to a view of metabolism in which enzyme concentrations are parameters, i.e., constant unless modulated by the control analyst. This is appropriate in cell free extracts or when gene expression is slower than observation.

It should be noted that MCA is not by necessity limited to intermediary metabolism but can also extend to DNA. To illustrate this we examine DNA supercoiling

in prokaryotes, known to be homeostatically controlled (Menzel and Gellert, 1983; Tse-Dinh, 1985; Westerhoff *et al.*, 1990). The linking number *Lk* is defined as the number of times that the two DNA strands of a covalently closed DNA molecule are intertwined. In *E. coli* it is subject to opposing enzyme activities: negative supercoiling by DNA gyrase and relaxation by topoisomerase I. The degree of supercoiling relates to a Gibbs energy potential. Taking the concentrations of DNA gyrase and topoisomerase I to be constant the control (metabolic control) of the enzymes on supercoiling was derived (Snoep *et al.*, 2000) to be as follows:

$$C^{\text{super}}_{v_{\text{gyr}}} = \frac{1}{-\epsilon^{v_{\text{gyr}}}_{\text{super}} + \epsilon^{v_{\text{top}}}_{\text{super}}} = -C^{\text{super}}_{v_{\text{top}}} \tag{28.1}$$

By 'super', the degree of negative supercoiling, by v_{gyr}, the activity of DNA gyrase, and by v_{top} topoisomerase I activity are indicated, respectively. The control coefficients (C) and elasticity coefficients (ε) are defined as usual (Fell, 1992). The equation shows that a high elasticity (sensitivity) of either (or both) of the enzymes with respect to the common intermediate (here DNA supercoiling) compromises the control either enzyme exerts on supercoiling. This is one face of homeostasis.

Hierarchical control analysis (*H*CA, Kahn & Westerhoff, 1991, Westerhoff *et al.*, 1990, 1998) deals with systems in which the enzyme activities themselves are regulated, either by gene expression or by signal transduction. Expression levels depending on DNA supercoiling is what is illustrated by Fig. 28.1. Also for such a hierarchical system the control coefficients (global control coefficients) can be expressed in elasticities. For the scheme in Fig. 28.1, assuming linear kinetics for mRNA and protein degradation, and taking transcription/translation to be independent for their products, the following expression could be derived (Snoep *et al.*, 2000):

$$C^{\text{super}}_{v_{\text{gyr}}} = \frac{1}{-\epsilon^{v_{\text{gyr}}}_{\text{super}} + \epsilon^{v_{\text{top}}}_{\text{super}} - \epsilon^{t_{\text{gyr}}}_{\text{super}} + \epsilon^{t_{\text{top}}}_{\text{super}}} = -C^{\text{super}}_{v_{\text{top}}} \tag{28.2}$$

The two additional elasticities reflect the sensitivity of gyrase and topoisomerase I transcription to supercoiling (t_{gyr} and t_{top} being the transcription/translation rates of respectively DNA gyrase and topoisomerase I). Realizing that gyrase expression is repressed (negative elasticity) and topoisomerase I expression is induced by supercoiling, comparison of eq. 28.2 to eq. 28.1 shows that in the hierarchical system control of topoisomerase I and gyrase is reduced as compared to the metabolic system. The variable gene expression is the other face of homeostasis.

Thus, the elasticities of the system provide one with a tool to quantify the contribution of metabolism and gene expression to homeostasis. The higher the absolute sum of the elasticities on a level, the higher the homeostatic control via that level. For a metabolic system to be stable both elasticities cannot be zero; there must be some sort of communication between the enzymes and the metabolite. In the simplest case the first enzyme should have a zero elasticity

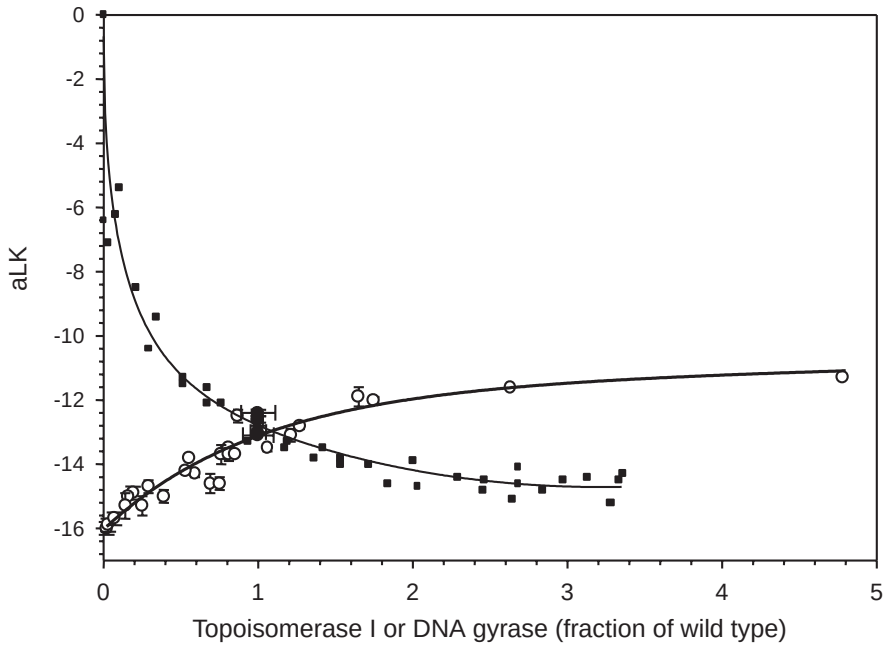

Fig. 28.2 aLk as a function of DNA gyrase or topoisomerase I activity. DNA gyrase (closed squares) or topoisomerase I (open circles) concentrations were manipulated using the mutant strains in which the native promoter is replaced by an IPTG inducible promoter.

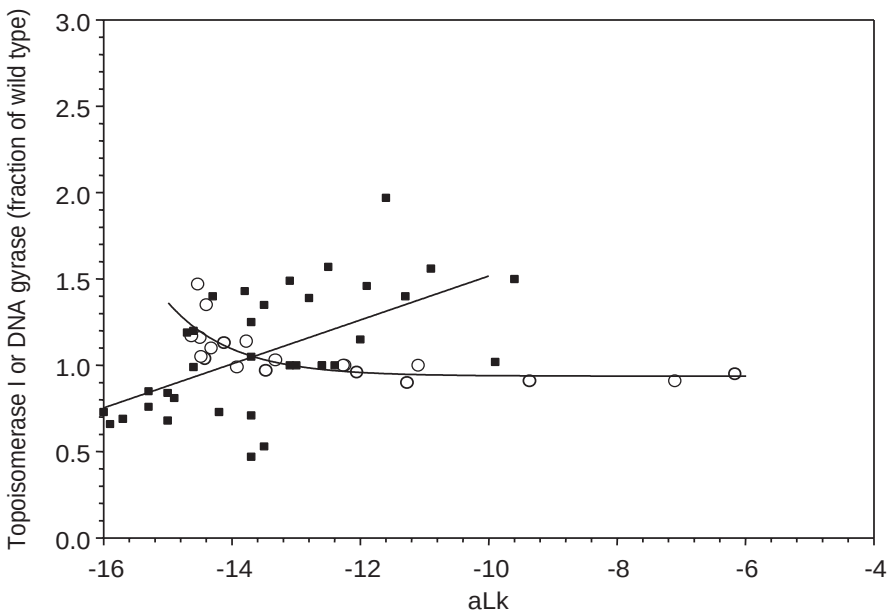

Fig. 28.3 DNA gyrase or topoisomerase I expression as a function of aLk. DNA gyrase (closed squares) concentrations were measured in cultures were topoisomerase I was modulated and topoisomerase I (open circles) concentrations were measured in cultures were DNA gyrase was modulated.

(irreversible, product insensitive enzyme) and the second enzyme should have an elasticity of 1 (linear kinetics with respect to the intermediate). In this simplest case of a metabolic system the sum of elasticities is 1 and the control of each enzyme on the intermediate is 1 (negative sign for topoisomerase I). The simplest form of gene expression control is a zero elasticity of gene expression with respect to supercoiling, i.e., expression being independent of supercoiling.

How can one now study such systems and determine the elasticities? Experimentally one could chose to replace the native promoter of a gene by an inducible promoter under control of an external substance. Thereby one changes the system in the sense that the elasticity of expression of the modulated enzyme with respect to supercoiling is zero in the mutant strain. The control coefficient determined in this way has been termed an inherent control coefficient (Jensen *et al.*, 1999) and is a special case of a co-response coefficient (Snoep *et al.*, 2000). In such a mutant strain, expression of the non-modulated enzyme is still sensitive to supercoiling and by measuring changes in its expression level this elasticity can be quantified. By repeating the same procedure for the other enzyme the elasticities of expression can be quantified and from the control coefficients measured in these systems one can then also deduce the sum of elasticities of the activities of both enzymes.

Experiments on the model system

Escherichia coli strains in which an IPTG inducible promoter controlled expression of DNA gyrase or topoisomerase I have been used to determine the co-response of gyrase and topoisomerase I with supercoiling and to measure the sensitivity of the expression of the non-modulated enzyme with respect to supercoiling.

The co-response (inherent control) of DNA gyrase and topoisomerase I with supercoiling was 0.17 and −0.13 respectively (Fig. 28.2). Also the elasticity of expression of DNA gyrase for supercoiling was higher than the elasticity of topoisomerase I, −1.4 and 0.5 respectively (Fig. 28.3). Together these coefficients can be used to calculate the metabolic and the global control coefficients of gyrase and topoisomerase I. The global control coefficient of gyrase was calculated to be 0.13 and the global control coefficient of topoisomerase I −0.12. Metabolic control coefficients of the two enzymes were calculated as 0.18 and −0.16, respectively. Finally the sum of elasticities on both levels can be calculated. The sum of the absolute values of the metabolic elasticities equalled 6 while the sum of the gene expression elasticities equalled 2.

Conclusions

Control coefficients can be used as indicators for homeostasis. Low concentration control coefficients indicate a strong homeostasis. This is reflected in the global control coefficients of DNA gyrase and topoisomerase I, the values between 0.12 and 0.13 indicating a strong homeostasis of DNA supercoiling. We have proposed to use elasticity coefficients as a measure for the extent of homeostatic control. The advantage of using elasticity coefficients is that they make it possible to understand where the homeostasis comes from in terms of enzyme characteristics. In addition the elasticity coefficients can be related to regulatory loops and thereby allow for a quantification of the importance of such a loop in the homeostasis. This method has been illustrated for the model system of DNA supercoiling in *E. coli*. Here the homeostasis can be divided in two levels, the metabolic level where the sensitivities of the enzyme activities with respect to supercoiling lead to a buffering of DNA supercoiling and secondly the gene-expression level where a variable gene expression of both DNA gyrase and topoisomerase I lead to homeostasis. In our model system (under the conditions used) the elasticities of the enzyme activities were three times as important as the regulatory loop via gene-expression modulation, as deduced from the sum of elasticities. To our knowledge this is the first case were the relative contribution of gene expression and enzyme activity to homeostasis has been quantified.

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