Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway

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Abstract The fact that information flows from DNA to RNA to protein to function suggests that regulation is 'hierarchical', i.e. dominated by regulation of gene expression. In the case of dominant regulation at the metabolic level, however, there is no quantitative relationship between mRNA levels and function. We here develop a method to quantitate the relative contributions of metabolic and hierarchical regulation. Applying this method to the glycolytic flux in three species of parasitic protists, we conclude that it is rarely regulated by gene expression alone. This casts strong doubts on whether transcriptome and proteome analysis suffices to assess biological function.

Key words: Metabolic control; Hierarchical control; Metabolic flux; Regulation of transcription

1. Introduction

The usually unspoken assumption underlying all genomic studies is that the expression of a gene at the mRNA level is a quantitative predictor for function. In short, an X-times higher signal at the Northern blot or gene chip level means X-times more protein and X-times more function. In particular, in the case of enzymatic reactions that are part of a metabolic pathway, this assumption may not reflect reality. At any time the rate of an enzymatic reaction is a function of the substrate(s), product(s), modifier(s), so-called metabolic properties and of gene expression, changing the concentration of the enzyme in question. Here we define 'metabolic', as all changes caused by concentrations of substrate(s), product(s) and modifier(s). The 'hierarchical' changes are those caused by change in enzyme concentration, via alterations in mRNA sequestration and intracellular localization and/or rates of whichever process, be it transcription, translation or degradation. Below, we consider regulation of steady-state fluxes through enzymes by either class of properties. We divide the change in the rate of an enzyme-catalyzed reaction, that is not directly affected by the external regulator, by the change in flux through the pathway to which the enzyme belongs. At steady state, the resulting ratio is 1, and distributed over hierarchical and metabolic regulation:

$$1 = \rho_h + \rho_m \tag{1}$$

ρ_h and ρ_m are the products of co-response coefficients and elasticity [1] quantifying the hierarchical (gene expression) and the metabolic component of the regulation, respectively. The hierarchical regulation coefficient is defined as the relative change in enzyme-catalyzed rate divided by the relative change in enzyme concentration (this factor usually equals 1 as expressed by the $\cong$ sign below) multiplied by the ratio of the change in enzyme concentration to the change in flux. Although this is not essential, all changes are here taken relative and small, such that the corresponding mathematical equation for the hierarchical regulation coefficient of an enzyme i is:

$$\rho_h = \frac{\partial \ln v_i}{\partial \ln e_i} \cdot \frac{d \ln e_i}{d \ln J} \cong \frac{d \ln e_i}{d \ln J} \tag{2}$$

J represents the pathway flux, e_i is the concentration of any enzyme i through which the flux runs, at a rate v_i. The metabolic component of the regulation is given by the change in enzyme rate divided by the change in concentration (X) of any of the varying metabolites around it, multiplied by the ratio of change in that concentration to the change in flux. This should be summed over all metabolites that change:

$$\rho_m = \sum_X \frac{\partial \ln v}{\partial \ln X} \cdot \frac{\partial \ln X}{\partial \ln J} \tag{3}$$

Graphically, the slope of a double logarithmic plot of the enzyme concentration (activity) versus the flux, which corresponds to a co-response coefficient [1], will give the hierarchical regulation coefficient, ρ_h. By virtue of the above relationship, the metabolic component can be obtained by subtraction of the ρ_h from 1 (100%). For full derivation see [2]. The regulation of each enzyme of a pathway can thus be compared with that of the others to provide a sophisticated picture of the regulation of the entire pathway. This type of analysis is complementary to metabolic control analysis [3,4], which considers pathways at constant gene expression or in long-term steady states after manipulation by genetic means.

2. Materials and methods

The species used and the methodology are described in references

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[5,6]. Further analysis was performed using Excel (Microsoft) software.

3. Results

This approach was applied to an experimental example that is particularly suited for such analysis: a study of well defined steady states concerning regulation and adaptation of the glycolytic pathway of three species of parasitic protists, *Trypanosoma brucei*, *Leishmania donovani* and *Trichomonas vaginalis*, growing on glucose [5,6]. Each species was grown in chemostats at approximately 0.2, 0.4, 0.6, 0.8 and 0.95 times the maximum growth rate under glucose limitation. At each steady state, the magnitudes of fluxes and the activities of most enzymes of the pathway were documented.

3.1. Enzyme activity and flux

Fig. 1 is a double logarithmic plot of enzyme activities versus the accompanying flux. 'Enzyme activity' is defined throughout as the in vitro rate at saturating substrate concentrations. The enzymes shown were selected to illustrate possible ways of regulation. Glycerol-3-phosphate dehydrogenase of *T. brucei*, and phosphoglucoseisomerase (PGI) of *L. donovani* showed completely metabolic regulation: whereas flux changed more than an order of magnitude, there was little change in activity of the enzyme. Therefore the ρ_h amounted to 0.0, hence ρ_m to 1.0. Apparently, the higher flux through these metabolic steps was entirely due to changes in metabolite concentrations; changes in expression level were irrelevant for the regulation of flux through this enzyme with increasing growth rate. This was also the case when glucose was supplied in excess and growth rate was limited by another nutrient (data not shown). Completely hierarchical regulation ($\rho_h = 1.0$) occurred in the case of malic enzyme of *L. donovani*.

Regulation can also be neither totally hierarchical nor totally metabolic. The extent to which metabolic flux through an enzyme is regulated hierarchically or metabolically differs between organisms and between enzymes. Regulation of the flux through the PGI and inorganic pyrophosphate-dependent phosphofructokinase (PPi-PFK) steps of *T. vaginalis* illustrates this. The average ρ_h of PPi-PFK (as calculated from the slope in the ln–ln plot) was close to two-thirds, one-third remaining for metabolic regulation. PGI in *T. vaginalis* was regulated hierarchically for only 25%, while other enzymes showed different distributions between 0 and 1 (Table 1).

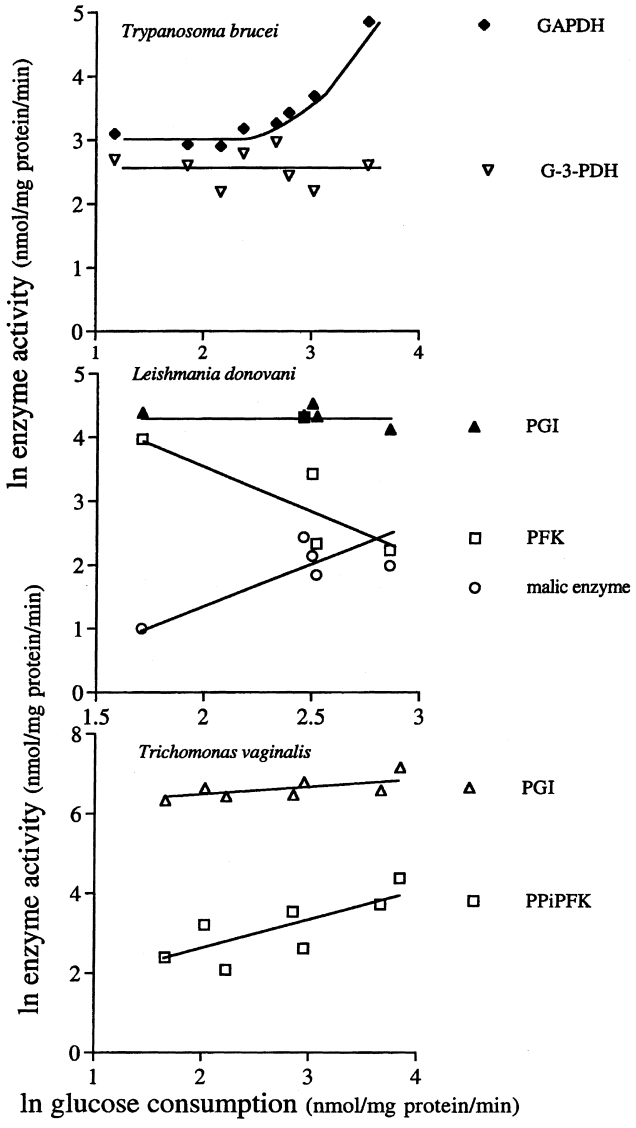

Fig. 1. Double logarithmic plot of enzyme activities, indicated by their abbreviations, against the flux that runs through them. Species are named in the upper part of each panel. Note that scales are different for each plot. Explanation in the text.

3.2. Shifts in regulation

Biology is complex and methods of analysis must take this into account: within a single organism, for a single enzyme, the method of regulation can shift as regulation proceeds. This was observed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *T. brucei*; a shift from metabolic regulation

Table 1
Relative abundance of four types of regulation

Species	Regulation type			
	supermetabolic ($\rho_h < -0.2$)	metabolic ($-0.2 < \rho_h < 0.2$)	shared ($0.2 < \rho_h < 0.8$)	hierarchical ($0.8 < \rho_h < 1.2$)
T. brucei	6	7	3	1
L. donovani	7	2	1	2
T. vaginalis	1	8	3	0

Numbers of enzymes that show negative hierarchical or supermetabolic, regulation ($\rho_h < -0.2$), metabolic regulation ($-0.2 < \rho_h < 0.2$), regulation shared between the hierarchical and metabolic routes ($0.2 < \rho_h < 0.8$) and hierarchical regulation ($0.8 < \rho_h < 1.2$). Super-hierarchical regulation (i.e. when enzyme activity increases more than proportionally as compared to the flux ($\rho_h > 1.2$) occurred only at the higher fluxes. When highest and second highest fluxes are compared it was found in eight out of 29 cases of *T. brucei* and *L. donovani* combined and in nine out of 12 for *T. vaginalis*.

at low dilution rates (average ρ_h close to 0) to overcompensating hierarchical control at high dilution rates (average ρ_h exceeding 1) was observed. It is as if the organism uses metabolic regulation to upregulate the GAPDH reaction until that mechanism is no longer sufficient and then invokes hierarchical regulation to achieve even higher fluxes through this step. The switch between the two types of regulation appeared to occur over a limited range of glucose consumption rates.

Thus far, hierarchical regulation analysis helped us to appreciate the relative importances of metabolic and hierarchical regulation. However, living systems can be more surprising than this. Negative hierarchical regulation at an average ρ_h of -0.4, hence a metabolic regulation of 1.6, was observed in the case of regulation of the flux through the PFK step in *L. donovani*. *Negative* hierarchical control was also noticed for several more enzymes of *T. brucei* and *L. donovani*. This may reflect the phenomenon that in the latter species, synthesis rates of some glycolytic enzymes are independent of growth rate. The lower activities at higher growth rates are caused by the enzyme being 'diluted' by other cell material [6].

The other extreme of regulation was also found, as exemplified by GAPDH in *T. brucei* at high glucose consumption rates. Here ρ_h becomes as high as 1.8, the metabolic regulation becoming negative at approximately -0.8. This we interpret as extensive positive adaptation by the organism; when it sees more glucose, it enhances the relevant gene-expression more than proportionately. Metabolic regulation then runs behind to the extent that it acts homeostatically.

3.3. Distribution of types of regulation

The above being mere examples of different scenarios of regulation, we did a more complete analysis for glycolytic enzymes in three organisms. The following types of regulation were found more than twice: largely metabolic regulation ($-0.2 < \rho_h < 0.2$), largely hierarchical regulation ($0.8 < \rho_h < 1.2$), dual regulation ($0.2 < \rho_h < 0.8$) and negative hierarchical regulation ($\rho_h < -0.2$) (Table 1). Positively adaptive hierarchical regulation ($1.2 < \rho_h$) occurred only after a switch in regulation at the higher rates of glucose consumption, as shown in the example of GAPDH of *T. brucei* (Fig. 1).

4. Discussion

Hierarchical regulation analysis yields a quantitative, phenomenological picture of regulatory processes in the cell. More importantly, it is a rational way to decide whether regulation occurs metabolically or through alterations in gene expression (i.e. hierarchically). In view of the enormous progress in possibilities of analyzing changes in gene expression levels, at least at the level of mRNA concentrations using hybridization arrays, one should hope that in most cases regulation is largely hierarchical. Then transcriptome and/or proteome studies should suffice to delineate regulation.

We here have shown that for a very common function (glycolysis) in not overly exotic organisms, regulation is rarely completely hierarchical. In fact it was mostly not even largely hierarchical. In essence, we found that the fluxes through steps in a metabolic pathway did not correlate proportionally with the concentrations of the corresponding biochemical enzymes. Of course, incomplete correlation between function (e.g. fluxes) and mRNA levels may occur even when regulation is largely hierarchical. Firstly, even at steady state, message levels did not correlate with enzyme activities in the three species used for this study [5,6]. Secondly, when one does not study the system at steady state, the enzyme concentrations may not yet have relaxed to their new steady-state values, whereas the mRNA concentrations have. This more trivial explanation is not relevant to steady-state chemostat cultures. Another explanation for the absence of correlation between mRNA levels and fluxes has been regulation at the posttranslational-modification level. Because we compared enzyme activities to fluxes, this cannot explain our findings either. We conclude that the both transcriptome and proteome may be vastly incomplete monitors of regulation of cell function. The metabolome [7,8] must also be taken in consideration in functional genomics.

That perhaps being a negative note, there are positive notes as well. Firstly, this paper shows a new and quantitative way to evaluate the importance of metabolic regulation by demonstrating that it equals 1 minus hierarchical regulation, provided that either type of regulation is expressed in terms of regulation coefficients. Secondly, it reveals that the extent to which regulation is hierarchical versus metabolic can vary with growth conditions of the organism. Consequently, it may enable one to find conditions where regulation is largely hierarchical and transcriptome/proteome analysis should be telling. When regulation is shown to be completely metabolic, there is also no need to search for regulatory mechanisms at the transcriptional or translational level. In addition, knowing that a particular enzyme is regulated largely metabolically may make it worthwhile to undertake the more tedious metabolic analyses, because the latter can then be focused on fewer enzymes.

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