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Adaptive Benefits of Storage Strategy and dual AMPK/TOR Signaling in Metabolic Stress Response

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Abstract

Cellular metabolism must ensure that supply of nutrient meets the biosynthetic and bioenergetic needs. Cells have therefore developed sophisticated signaling and regulatory pathways in order to cope with dynamic fluctuations of both resource and demand and to regulate accordingly diverse anabolic and catabolic processes. Intriguingly, these pathways are organized around a relatively small number of regulatory hubs, such as the highly-conserved AMPK and TOR kinase families in eukaryotic cells. Here, the global metabolic adaptations upon dynamic environment are investigated using a prototypical model of regulated metabolism. In this model, the optimal enzyme profile as well as the underlying regulatory architecture are identified by combining perturbation and evolutionary methods. The results reveal the existence of distinct classes of adaptive strategies, which differ in the management of storage reserve depending on the intensity of the stress and in the regulation of ATP-producing reaction depending on the nature of the stress. The regulatory architecture that optimally implements these adaptive features is characterized by a crosstalk between two specialized signaling pathways, which bears close similarities with the sensing and regulatory properties of AMPK and TOR pathways.

Introduction

To cope with environmental changes that impact their metabolism, living cells have evolved adaptive strategies consisting in sensing their extracellular or intracellular environment and regulating accordingly the activity of enzymes catalyzing metabolic reaction pathways. These strategic tasks involve only a few signaling pathways in spite of the huge number of enzyme-catalyzed metabolic pathways. In eukaryotes, the highly conserved AMPK (AMP-activated kinase) and TOR (target of rapamycin) families of protein kinase have crucial and numerous roles in nutrient and energy sensing, and in governing metabolic adaptations by regulating the expression and post-translational modifications of many metabolic enzymes [1–3]. Mammalian AMPK and its yeast and plant homologs Snf1 and SnRK1 are prone to be activated, allosterically or through phosphorylation, upon intracellular increases of AMP or ADP levels [4–6]. In turn, AMPK/Snf1/SnRK1 kinases tend to switch off anabolic pathways, including the biosynthesis of proteins, ribosomal RNA, carbohydrates or lipids while promoting their degradation through autophagy and fatty acid oxidation [7]. For its part, the TOR pathway is rather sensitive to intracellular levels of metabolites, especially amino acids, and

promotes growth by activating regulating biosynthetic pathways at the level of both transcriptional and translational machinery [8–10]. Besides their opposite roles in regulating biosynthetic pathways, both signaling pathways nevertheless share the same inclination to activate certain processes such as glycolysis or mitochondrial oxidative metabolism. For the latter, TOR promotes PGC-1 α [11], 4EBP dependent translational regulation [12] or TCA enzymes such as Glu dehydrogenase [13], and AMPK mediates as well the activation of mitochondrial enzymes mainly through pathways converging to PCG1 α /p [14, 15].

The crosstalk between AMPK and TOR signaling in sensing various intracellular cues and in regulating diverse anabolic and catabolic pathways raises a number of theoretical issues. The issue of intracellular sensing raises a difficult problem as these sensors are embedded into a global feedback architecture [16, 17]. As well, regulatory logic has been mainly studied for unbranched metabolic pathways [18–21] but much less for coupled metabolic pathways that both cooperate and compete for the utilization of internal resources. Besides the detailed schemes of sensing and regulatory mechanisms, several general questions arise about the adaptive logic of cell metabolism: How do signaling and regulatory strategies depend on the nature, frequency, duration, amplitude or randomness of environmental perturbations? What are the minimal requirements and the precise mechanisms that confer an adaptive benefit upon storage metabolism? The present study aims to address most of these issues through a minimal modeling approach.

Diverse computational modeling approaches have been developed to study the regulation of cell metabolism [22]. These approaches are generally based on a dual-level description made of a metabolic reaction network and an enzyme regulatory network. First, constraint-based stoichiometric models of genome-scale metabolic reaction network use steady-state assumptions and do not provide information on the enzymatic concentrations. Nevertheless, several extensions have attempted to overcome these limitations by incorporating a description of gene regulation [23], by considering enzyme costs and capacity constraints [24], by performing timescale separation hypothesis [24], or by using sensitivity analysis [25]. Second, metabolic control analysis is a powerful framework to study the response properties of complex metabolic systems to small changes of the kinetic parameters, which can be used to derive the optimal linear feedback regulation to static perturbation of steady state [26], and can be extended to the cases of non-steady state trajectories [27] or of time-dependent changes of kinetic parameters [28]. Although these two main modeling frameworks are well-adapted to determine optimal flux balance in detailed metabolic reaction networks, they remain dependent on steady-state or quasi-steady-state assumptions or on small perturbation approximations. A third approach consists in using simplified models depicting generic motifs (unbranched or cyclic pathways) or a prototypical metabolism, which allows to study regulation in simple resource allocation problem such as the switch from one to another substrate [30, 37], the switch between respiratory and fermentation metabolism [31], or the evolution of regulatory complexity [29]. The total number of kinetic parameters in these models is usually low enough to allow (i) for extensive parameter space exploration or (ii) for parameter optimization through evolutionary computation techniques, without necessarily requiring additional assumptions of steady state or of small enough environmental fluctuations.

In this paper, minimal modeling and evolutionary computation are exploited to investigate the regulated coordination of catabolic and anabolic processes, and to decipher the logic underlying the universal sensing and regulatory features of TOR and AMPK signaling pathway. To this purpose, we introduce a coarse-grained model of cell metabolism that recapitulates the main catabolic and anabolic pathways. Steady-state and perturbation analysis are first performed to identify the regulatory logic in response

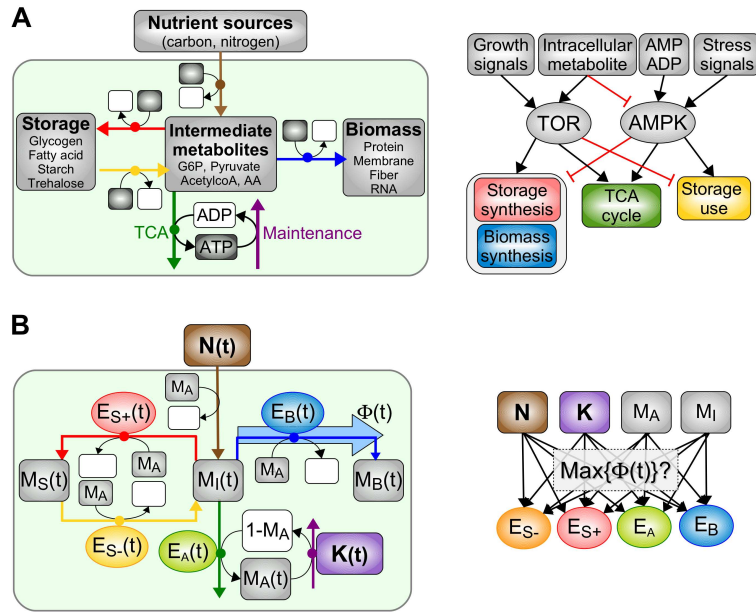


Figure 1: **Coarse-grained description of cell metabolism.** (A) Main anabolic and catabolic metabolic pathways regulated by TOR and AMPK/Snf1 signaling pathways. (B) Corresponding model including metabolic variable M_i , enzyme variable E_i and varying resource N and demand K , organized into a metabolic network (left) and a signaling/regulatory network (right).

to very slow or very small perturbations. Evolutionary computation is then applied to investigate adaptive strategy to a large range of perturbation amplitude and frequency, and to obtain the optimal enzyme time course and regulatory parameters. The results and the closing discussion emphasize the critical roles of storage metabolism, internal sensing and regulatory crosstalk, for metabolic adaptation to dynamic and complex environments.

Methods

We consider a coarse-grained description of cell metabolism where nutrients are imported and catabolized into intermediate metabolites that can be either oxidized through the TCA cycle to produce ATP or utilized as precursors to build storage or biomass materials. In turn, ATP fuels most of the import, maintenance and biosynthetic processes (Fig. 1A). Each of these coarse-grained processes are based on a chain of reactions that is regulated by a pool of enzyme and is characterized with a global energy budget in terms of ATP cost or gain. Such schematic model of regulated metabolism can be translated into a biochemical reaction rate model (Fig. 1B and Table 1) where each macroprocess is modeled by a single reaction catalyzed by a single enzyme and consumes a given amount of ATP. The model therefore comprises different classes of variables and parameters : (i) metabolic variables M_j where $j = I$ for intermediate, $j = S$ for storage, $j = B$ for biomass and $j = A$ for ATP; (ii) regulatory variables E_j for the concentrations of enzymes catalyzing the j th reaction ($j = A, B, S_+, S_-, T, M$); (iii) contextual/environmental variables, N for extracellular nutrient concentration and K for the energy demand; (iv) budget parameters k_j for the cost or gain in ATP of the j th reaction.

The time evolution of metabolic variables follows the differential equation system:

$$\frac{d\vec{M}}{dt} = \vec{F}(\vec{M}(t), \vec{E}(t), N(t), K(t)) \quad (1)$$

where \vec{F} is given in Table 1. It is to mention that two reactions are assumed to be catalyzed by constant enzyme level: (i) the nutrient transport reaction rate that is described by a non-regulated anisotropic diffusion process and (ii) the maintenance (i.e., housekeeping) reaction that is described as a zeroth order reaction and depends on the concentration of storage and enzyme (Table 1).

Table 1: **Biochemical reactions and parameter values.**

Nutrient transport	$N + k_T M_A + E_T \xleftrightarrow{*} M_I + k_T(1 - M_A) + E_T$ $v_1 = E_T M_A \mathcal{H}(N - M_I)$
ATP production	$M_I + k_A(1 - M_A) + E_A \rightarrow k_A M_A + E_A$ $v_2 = E_A(1 - M_A) M_I$
Biomass production	$M_I + k_B M_A + E_B \rightarrow M_B + k_B(1 - M_A) + E_B$ $v_3 = E_B M_A M_I$
Storage production	$M_I + k_{S+} M_A + E_{S+} \rightarrow M_S + k_{S+}(1 - M_A) + E_{S+}$ $v_4 = E_{S+} M_A M_I$
Storage degradation	$M_S + k_{S-} M_A + E_{S-} \rightarrow M_I + k_{S-}(1 - M_A) + E_{S-}$ $v_5 = E_{S-} M_A M_S$
Maintenance reaction	$M_A \rightarrow (1 - M_A)$ $v_6 = K_0 + K_S M_S + K_E \sum_i E_i$
Parameters	$k_A = 30; k_B = 5; k_T = 1; k_{S+} = 4; k_{S-} = 1$ $K_0 = 1; K_S = 0.01; K_E = 1; E_T = 0.5$
Rate equations	$\dot{M}_A = -k_T v_1 + k_A v_2 - k_B v_3 - k_{S+} v_4 - k_{S-} v_5 - v_6$ $\dot{M}_I = v_1 - v_2 - v_3 + v_5 - v_4; \dot{M}_S = v_4 - v_5; \dot{M}_B = v_3$

Reaction rates are based on first-order rate law, except the 0th order maintenance reaction rate. * indicates that the reversible reaction occurs only for $N > M_I$ with an Heavyside function \mathcal{H} in the rate law. $1 - M_A$ denotes the converted form of M_A with a unit total pool concentration.

The sources of nonstationarity in the model are of two sorts : the changes in extra-cellular nutrient levels $N(t)$ and the changes in energy demand $K(t)$ as ATP-consuming cellular functions (stress management, motility, morphological changes...) are prone to be sensitive to environmental changes and transient in time. For simplicity, we consider sinusoidal variations of $N(t)$ and $K(t)$:

$$N(t) = N_0 - \frac{(a_N \cos(\omega_N t)) - 1}{2} \quad (2)$$

$$K(t) = K_0 + \frac{(a_K \cos(\omega_K t)) - 1}{2} \quad (3)$$

where $a_{N,K}$ are the perturbation amplitudes from such basal levels N_0 and K_0 and $\omega_{N,K}$ are the perturbation frequencies. Given these nonstationary conditions, the optimization criterion for metabolic fitness is the time-averaged biomass production rate in the permanent regime:

$$\Phi = \frac{1}{T} \int_{t_0}^{t_0+T} E_B(t) M_I(t) M_A(t) dt \quad (4)$$

$$M_i(t_0 + T) = M_i(t_0), \quad \{i = I, A\}$$

where $[t_0, t_0 + T]$ is the sampling time window and $T = 2\pi/\omega$.

Metabolic parameter values

The prototypical model of metabolism depicted in Fig. 1 is not specific to a particular organism and does not take into account the diversity of nutrient sources, storage compounds and functional biomass compounds (e.g., DNA, RNA, proteins...). As a result, model parameters values are not necessarily related with known reaction rates and stoichiometries associated with a selected metabolic pathway. Nevertheless, the choice of parameter values has been made to match the order of magnitudes of some global or averaged biological quantities (Table 1). Parameter values are dependent on the concentration and time unit chosen. The assumption that the total concentration of the pool of adenylyl phosphate is constant and equal to 1 defines the unit of concentration. Because the experimentally measured value is of the order of 10mM (1mmol/L) and the molecular weight of ATP is 507g.mol⁻¹, the concentration unit is set to 0.5g/L. The unit of time is given by the arbitrary choice that the basal decay rate of ATP is unit-normalized with $K_0 = 1$. The biological value of the basal consumption rate of ATP can be approximately derived from the respiratory rates $J_{ATP} \sim 50\text{mM}\cdot\text{min}^{-1}$ measured in yeast cells in the stationary phase [33]. Given the concentration unit defined above and an ATP:ADP ratio of about $\sim 5 : 1$, the consumption rate would be 6min^{-1} which corresponds to a time unit of 10s. This upper bound of ATP lifetime is consistent with the measured values of ATP turnover time of the magnitude of second in diverse growth conditions and species [32].

Parameters for ATP production and consumption are chosen from the global gain or cost of ATP associated with a whole metabolic process. The value for ATP gain associated with TCA cycle is set to $k_A = 30$, which is similar to the order of magnitude of 25g of ATP produced through the oxidation of 1g of acetyl COA (both metabolites have a similar molar mass). The value for ATP consumption associated with biomass production is set to $k_B = 5$ as the minimal energy cost for protein synthesis is the 5 ATP hydrolyzed for each peptide bond formed, assuming that the molar mass of peptide is similar to that of ATP and neglecting other biosynthetic costs. The ATP cost for the whole process of storage production, maintenance and consumption depends on the type of storage compounds. We use the following arbitrary values $k_{S_+} = 4$, $k_{S_-} = 1$, $K_S = 0.01$ and have checked a posteriori that storage content is lower than 10 times the adenylyl phosphate content $M_s < 10$, as starch or glycogen contents is usually limited to a maximum of a few percent of cell mass whereas ATP content of the magnitude of 0.1%. The parameter value E_T for nutrient import is based on the glucose import rate measured in budding yeast. Depending on the extracellular glucose concentration and the type of hexose transporter involved, glucose import rate can be estimated between 10 and 100min^{-1} [34], which translates into $0.5 < E_T < 5$ for the concentration and time units defined above.

Regulatory parameter optimization

The search for regulatory parameters that shapes $\vec{E}(t)$ so as to maximizes the flux Φ in dynamic environments requires to use parameter optimization techniques. Perturbation methods are well adapted in the case of small enough environmental fluctuations. Environmental, enzyme and metabolic variables can be expanded up to first order $x = x_0 + \epsilon x_1$ ($x = M_i, E_i, N, K$) where first-order terms are real trigonometric polynomial functions:

$$x_1 = c_i + \sum_{i=1}^n a_i \cos(i\theta) + b_i \sin(i\theta) \quad (5)$$

where $n = 1$ for $x = E_i, N, K$ and otherwise undefined. By substituting Eq.5 into Eq.1 and Eq.4, the vector field \vec{F} and the objective function Φ are expanded in power series

of ε , leading to a hierarchy of equations for \vec{M} and Φ that can be solved recursively by using a formal calculus software. To the 0th order in ε , the steady state condition $\vec{F}_0(\vec{M}_0, \vec{E}_0, \{N, K\}_0) = \vec{0}$ allows \vec{M}_0 to be expressed as a function of \vec{E}_0 and to be substituted into Φ_0 . Optimal enzyme parameters \vec{E}_0 are obtained by finding the single local maximum of Φ_0 that satisfies $\nabla \Phi_0(\vec{E}_0) = 0$ and $\nabla^2 \Phi_0(\vec{E}_0) > 0$. As well, solution of the linear equations at the following l th orders $\vec{M}_l = \vec{F}_l$ allows to find asymptotic time-dependent solutions $\vec{M}_l(t)$ as a function of trigonometric polynomial coefficients a , b and c of \vec{E}_l , while the optimized coefficient are obtained again by maximizing Φ_l .

In the case of large fluctuations $N(t)$ and $K(t)$, perturbation method is substituted by evolution strategy optimization technique that is a class of evolutionary algorithm [35]. The optimization criterion is the maximization of the metabolic flux Φ given by Eq.4 whereas the termination criterion depends on both the number of generation and a measure of evolution convergence.

Results

Steady-state metabolic adaptations: ATP homeostasis and metabolic collapse

In stationary condition defined by constant levels of nutrient N_0 , energy demand K_0 and enzymes \vec{E}_0 , a stable metabolic state corresponds to a fixed point of Eq.1 with non-negative value of nutrients, metabolites, storage, ATP and ADP. However, for some range of values of N_0 , K_0 and \vec{E}_0 , a stable metabolic state may not exist, such that all phase space trajectories drift toward the region of negative ATP level ($M_A < 0$), in which case metabolic death occurs. As mentioned in Methods section, an optimal stable metabolic steady state is defined for enzyme parameters \vec{E}_0 that maximize the metabolic flux Φ for any values N_0 and K_0 (Fig. 2). The optimal metabolic flux Φ decreases with decreasing values of N_0 and increasing values K_0 to $\Phi = 0$ at a threshold value $N_{0,c}(K_0)$ and $K_{0,c}(N_0)$ (Fig. 2A) beyond which metabolic death always occurs. This stress-induced decrease of Φ is paralleled with a decay of biomass production enzyme $E_{0,B}$ to 0 (Fig. 2B), while the ATP production enzyme $E_{0,A}$ is either slightly increased or decreased depending on whether it is a nutrient stress or an energy stress, respectively (Fig. 2C). In this result, the smaller regulation of $E_{0,A}$ compared to that of $E_{0,B}$ reflects the imperative need to maintain relatively constant and high levels of ATP for survival at the expense of a much reduced biomass production and flux (Fig. 2D). Furthermore, the opposite regulation of $E_{0,A}$ between the two types of stress reflects the fact that the many ATP-producing and ATP-consuming reactions can be differentially affected by the two stress types, such that stress-specific and finely-tuned regulation are required to reestablish ATP homeostasis. In contrast with these subtle mechanisms of ATP homeostasis, the optimal level of internal metabolites M_I roughly scales with external nutrients N_0 (Fig. 2E). Finally, an expected feature of the optimal steady state is the absence of storage enzymes $E_{0,S+/-} = 0$ as the processes of production, maintenance and degradation of storage generate metabolic costs in ATP and enzymes and any profits in stationary conditions. Note that even for the optimal enzyme parameters, a stable fixed point coexists with a saddle fixed point (Fig. 2F), such that transition to death can arise at the threshold condition $K_{0,c}$ and $N_{0,c}$ through a saddle-node bifurcation but also through transient perturbations, which is a critical feature for further investigations of the effect of dynamic fluctuations. Quantitatively, the value $\Phi \sim 0.12$ obtained for $N_0 = 1$ and the nutrient threshold of $N_0 = 0.3$ corresponds to a doubling time of ~ 10 h for an external glucose concentration of 25mM, which matches the order of magnitude

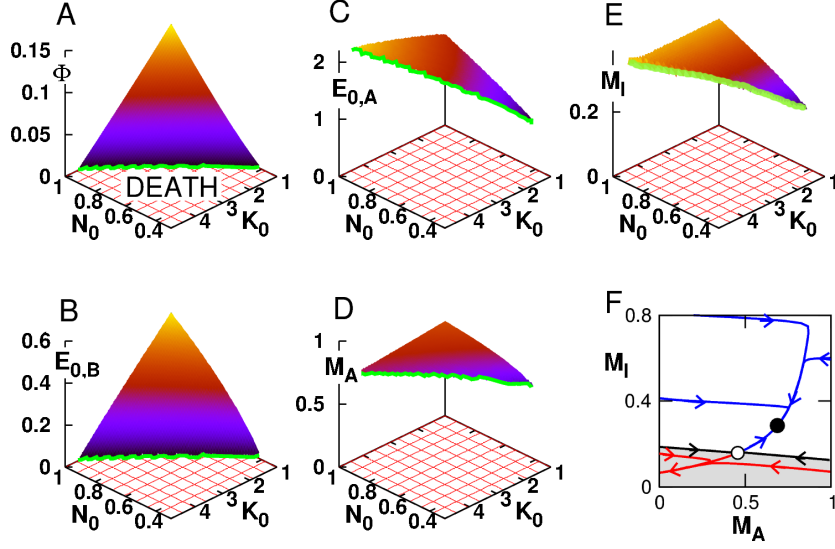


Figure 2: **Optimal steady-state solutions.** Properties of the optimal solution as a function of stationary parameters N_0 and K_0 : (A) Flux Φ where green line corresponds to $\Phi = 0$; (B-C) Stationary enzyme level $E_{0,B}$ and $E_{0,A}$; (D,E) Stable fixed point coordinate M_A and M_I ; (F) Example of phase space portrait and trajectories for $N_0 = 0.8$ and $K_0 = 2$ where black and white circles indicate stable and unstable fixed points and the black line separates the viability domain (white) and metabolic collapse domain (gray).

of experimental values [36].

Dynamic metabolic adaptations: just-in-time and storage strategies

After having characterized the main features of the optimal metabolic steady state, the following step is to search for optimal enzyme profiles in response to nonstationary environmental conditions such as oscillations of $N(t)$ and $K(t)$ of amplitudes $a_{N,K}$ given by Eq.2 (Fig. 3A). For simplicity, we assume a sinusoidal shape for $\vec{E}(t)$

$$E_i(t) = e_{0,i}(1 + a_i \cos(\omega_{N,K}t + \varphi_i)) \quad (6)$$

The optimal values for $e_{0,i}$, a_i and φ_i can first be derived for small amplitude oscillations a_i by using a perturbation method, and then obtained for any perturbation amplitude by using evolutionary methods (see Methods section). The results depicted in Fig. 3B shows that the optimal solutions are well predicted by the perturbation approach, up to relatively large stress amplitudes $a_{N,K}$, which can be explained by the almost linear relation between the metabolic flux and the parameter perturbation (Fig. 2A). For these optimal solutions, the enzyme oscillations display an increasing amplitude with perturbation amplitude and are in phase or antiphase with the perturbation depending on whether $dE_{0,i}/dN$ and $dE_{0,i}/dK$ are positive or negative in (Fig. 2B,C). The in-phase or anti-phase relationship between enzyme and perturbation oscillations is related with the assumption of a low perturbation frequency $\omega_{N,K} = 0.01$ (i.e, dimensionalized period of $T = 100\text{mn}$) that is much smaller than the natural frequency ω_0 of the metabolic system, while phase shifts would occur for $\omega_{N,K} \sim \omega_0$. This strategy bears

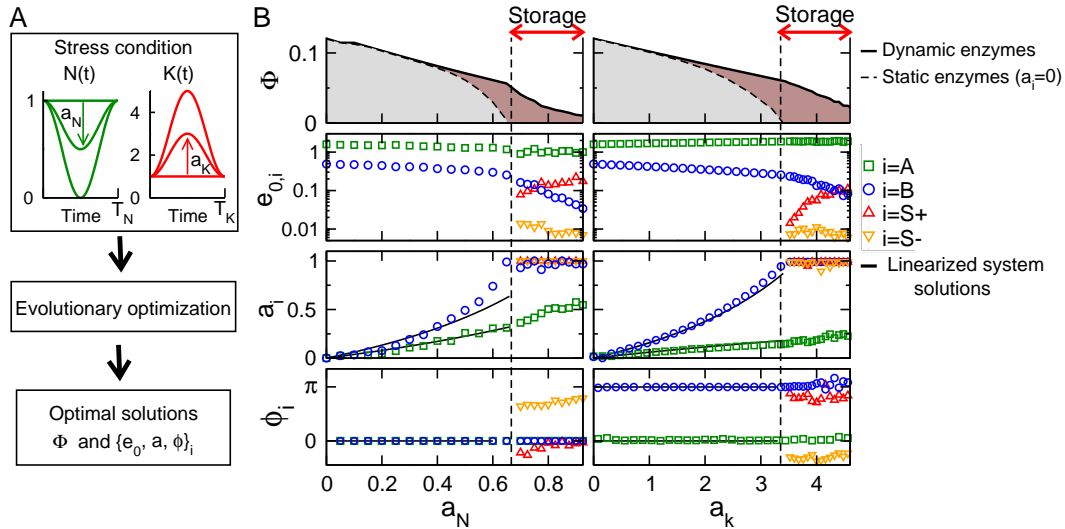


Figure 3: **Optimal enzyme profile in presence of time-dependent nutrient or energy stress.** (A) Simulation protocol to obtain optimal enzyme profile as a function of the stress condition. (B) Optimal solutions as a function of stress type and amplitude reveals the existence of storage-based solutions for large enough amplitude. Upper panel: Metabolic flux Φ , mean enzyme level $e_{0,i}$, amplitude of enzyme oscillations a_i , phase of enzyme oscillations φ_i . Optimal enzyme profile obtained with evolution methods (colored point) are compared with small amplitude solutions obtained with perturbation methods (continuous lines).

similarities with so-called just-in-time or lean manufacturing strategy for production lines as enzymes must be produced and activated at the right time by maximizing the use of rate-limiting substrates while avoiding accumulating or stored parts. However, above some critical perturbation amplitude $a_{i,c} > a_{i,c}$ ($i = N, K$), the optimal solutions display qualitatively different properties characterized by a tight regulation of storage production and degradation ($e_{0,S} > 0$, $a_{S+}, a_{S-} = 1$ and $\varphi_{S+} \sim \varphi_{S-} + \pi$).

These storage-based solutions occur for a given frequency range of oscillatory perturbations (Fig. 4A,B). The upper-bound frequency coincides with the undamped natural (also cutoff) frequency ω_0 of the low-pass second-order filter associated with the metabolic system $\{M_A, M_I\}$ linearized around the steady state $N_0 = k_0 = 1$. The storage strategy thus confers a fitness benefit for slow variations of $N(t)$ or $K(t)$ below the thresholds $N_{0,c}$ or $K_{0,c}$, which would not be filtered out and would induce metabolic death in the absence of slow storage cycles. In turn, the lower-bound frequency indicates that a too long stress requires to be anticipated with high storage reserves that comes with an unbearable cost and death. As a result, the precise values of this bound depends on the actual cost of storage production and maintenance.

The mechanism through which the accumulation and degradation of storage material buffer out slow environmental fluctuations can be captured by the low-pass filter component in presence of stationary levels of enzymes $E_i(t) = E_{0,i}$ (Fig. 4C). The dominant cutoff frequency coincides with the environmental frequency for which the system has been optimized. The optimal profile of the enzymatic variables and the corresponding time course for metabolic variables depicted in Fig. 4D illustrate how the optimal solution coincides with a temporal management of storage material, in order to ensure that M_A and M_I remain in the viability domain (shown in Fig. 2F) so as to avoid metabolic collapse.

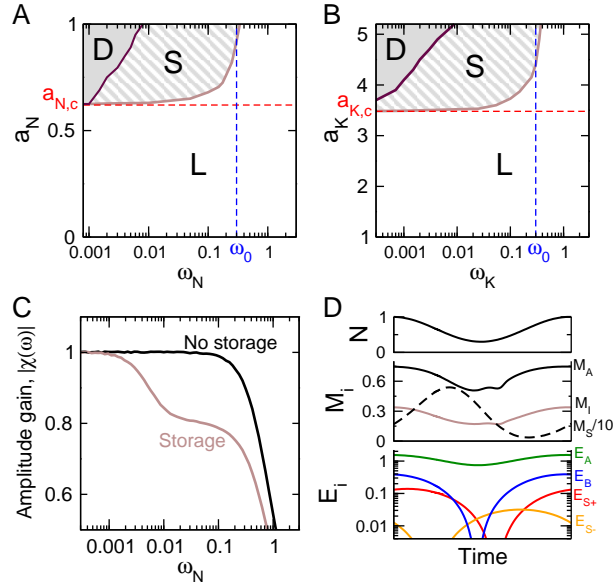


Figure 4: **Storage strategy.** (A,B) Distinct classes of optimal solutions (S: Storage; L: lean / no storage; D: Death) as a function of the type, the amplitude and the frequency of the stress condition. (C) Amplitude gain as a function of perturbation frequency in presence of static enzyme with and without storage reveals specific low-pass filtering properties. (D) Timecourse of metabolic and enzymatic variable illustrated for an optimal solution with nutrient stress $\omega_N = 0.01$ and $a_N = 0.75$.

Optimal signaling and regulation for dynamic adaptations to metabolic stress

The optimal oscillatory profiles of enzyme obtained for various nonstationary conditions provide guidance on the manner how these enzymes would be optimally regulated by specific signaling cues. For instance, the optimal phases of enzyme oscillations with respect to signal oscillations (see low panels of Fig. 3) are expected to predict whether these enzymes would be positively or negatively regulated by the signaling pathways sensitive to these signals. It remains however difficult to foresee which signaling cues and how many signaling pathways are required to regulate metabolism in an optimal manner. To address these issues, the metabolic network model given by Eq.1 is supplemented by a minimal description of the signaling pathways that regulate the time evolution of enzyme concentration :

$$\tau_i \frac{dE_i}{dt} = \mu_i \prod_{j=1}^{N_Y} f_{ij}(Y_j) - E_i \quad (7)$$

where Y_j is the signal input that can depend on any environmental or metabolic variables, and N_Y is the number of signaling pathway. The regulation function f_{ij} is described by a Hill function:

$$f_{ij}(x) = \frac{1 + \lambda_{ij} \left(\frac{x}{\theta_{ij}}\right)^{n_H}}{1 + \left(\frac{x}{\theta_{ij}}\right)^{n_H}} \quad (8)$$

where μ_i is the basal activation rate of E_i , λ_{ij} the Y_j -dependent activation (> 1) or inactivation (< 1) rate of E_i by Y_j , θ_{ij} the regulatory threshold (the inflection point of the response curve for $n_H = 2$), and n_H is the Hill coefficient or slope factor that

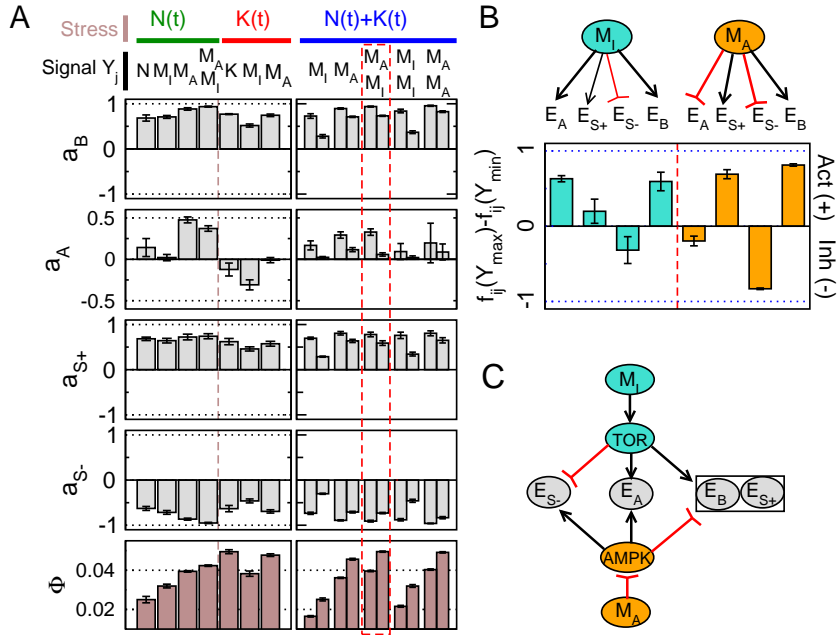


Figure 5: **Optimal signaling and regulatory pattern of enzyme in response to single or combined stress conditions.** (A) Enzyme amplitude (Eq. 9): average and variance values computed for the 20 better solutions over 40 evolutionary runs. Evolutionary optimization is made on the regulatory parameters μ_i , λ_{ij} and θ_{ij} for two distinct and combined stress conditions ($N(t)$: $N_0 = 1$, $\omega_N = 0.01$, $a_N = 0.7$, $a_K = 0$; $K(t)$: $N_0 = 1$, $\omega_N = 0.01$, $a_N = 0.$, $a_K = 3.5$) and for various signals. For optimization in combined stress conditions (right panel), left versus right bars corresponds to enzyme amplitudes measured when exposed to a single stress, $N(t)$ (left) or $K(t)$ (right). (B) Optimal regulatory parameters $f_{ij}(Y_{max}) - f_{ij}(Y_{min})$ represented as activatory or inhibitory regulations in presence of two signaling pathway and combined stress conditions (see dashed rectangle of (A)). (C) Corresponding regulatory scheme by assuming the existence of AMPK-like and TOR-like regulatory proteins.

is set to 2. Evolutionary optimization technique is applied to determine the regulatory parameters μ_i , λ_{ij} and θ_{ij} that maximizes the flux. We use constant value $\tau_i = 1$, which would correspond to a rapid mode of regulation (e.g, post-translational), since, anyway, optimization leads to minimize τ_i in the absence of synthesis and degradation costs for enzymes. To compare the optimal solutions obtained in the cases of oscillatory versus regulated enzymes, a generic quantity for enzyme amplitude is defined as,

$$a_i = \cos(\varphi) \frac{E_i^M - E_i^m}{E_i^M + E_i^m} \quad (9)$$

where $\varphi = 2\pi(t'_{E_i} - t'_{K,N})/T$ corresponds to the phase of the enzyme response (t'_x is the time of the maximum of $x(t)$). Optimization is first performed in presence of a single stress conditions ($N(t)$ or $K(t)$ with $\omega_{N,K} = 0.01$) and a single signaling pathway $N_Y = 1$ where Y_1 is a function of N , K , M_I or M_A (Fig.5A, left panels). The sensing functions are $Y(N) = N$, $Y(M_I) = M_I$, $Y(K) = 5 - K$ and $Y(M_A) = 0.1 M_A / (1 - M_A)$ and have been chosen to display similar maximal and minimal values for Y given the stress intensities considered here.

Irrespective to the signaling cue, a stress associated with low levels of N , M_I or M_A or high levels of K induces an inhibition of enzymes E_{S+} , E_B and activation of E_{S-} ,

while it induces either an activation or an inactivation of E_A depending on whether it is an energy or a nutrient stress, respectively (Fig.5A, left panels). These tendencies are consistent with the optimal oscillatory pattern of enzyme (see Fig.3). However, both the strengths of regulation and the metabolic flux Φ slightly depend on the type of signaling cues, which presumably reflects differences in the periodic time profile of $Y_j(t)$ which can be more or less sinusoidal or distorted. The result that Φ systematically augments by increasing the hill coefficient n_H (result not shown) or by increasing the number of signaling pathways N_Y is consistent with the notion that signaling complexity improves metabolic fitness through a refined control of enzyme time course in the absence of costs associated with increases of N_Y or n_H .

In the case where a single signaling pathway is optimized to maximize the sum of the flux for the two stress conditions (Fig.5A, right panels), the metabolic flux is decreased from 10% (for M_A) to 40% (for M_I) compared to the case where optimization is done for each stress condition separately. This result reflects the property that optimal regulation of E_A depends on the stress type, giving rise to a compromise solution of intermediate regulation of E_A . In contrast, optimization with two signaling pathways allows to recover the optimal fluxes obtained for each stress condition optimized separately with a single signaling pathway. This dual signaling and regulatory scheme shows a clear division of the sensing and regulatory task as the signaling sensitive to M_I inhibits E_A while the pathways sensitive to M_A activates E_A (Fig.5B), which is reminiscent to the acknowledged pattern of regulation by AMPK and TOR (Compare Fig.5C and Fig.1A). Besides their opposite regulation of E_A , the two signaling pathways also differ in the regulatory strength of storage enzyme, which also suggest a division of task based on the survival-growth dichotomy. The M_A -sensitive pathway is prone to lead to drastic metabolic adaptation upon severe stress, while the M_I -signaling pathway would rather achieve a more graded response to optimize the metabolic growth rate.

To summarize, the crosstalk between several signaling and regulatory pathways confers fitness advantages by refining the time profile of respective enzymes, but also by allowing a distribution of tasks when coping with different stress types and intensities.

Discussion

The analysis of a coarse-grained model of cell metabolism reveals distinct adaptive strategies in changing environments, depending on the nature, the amplitude, and the timescale of environmental changes. In line with previous studies, adaptation to small environmental fluctuations only requires to be compensated in time by dynamically reallocating the enzyme resources [37, 38] by analogy with just-in-time manufacturing strategies [18]. In contrast, metabolic adaptation to large environmental fluctuations involves storage management's pathways in order to buffer out these fluctuations and protect cells against detrimental outcomes for survival. The buffer effect relies on a slow storage degradation process, providing a low-pass filtering's property to the metabolic system. In this process, a tight regulation of the storage's production and degradation is of critical importance to minimize the cost of production and of maintenance of storage material. A typical example of such adaptive mechanism is the regulation of starch, a major form of stored carbohydrate in plants: starch is accumulated during the day and remobilized at night at a rate which depends on the night length to support continued respiration [39]. In fact, different storage compounds may exhibit differential capacities in coping with rapid or slow changes of their environment, depending on the energetic and temporal constraints associated with their production, transport, reactivity, and degradation. Carbohydrates, for instance, are energy's stores less concentrated than triacylglycerols, but are more rapidly mobilized. The specific roles of glycogen and trehalose during the diauxic shift response and the quiescence starvation response in yeast

further suggest the existence of distinct and combined storage strategies depending on the mode of production and reactivity of storage compounds [40]. Finally, proteins and other macromolecular complexes also constitutes large reserves of recyclable material that can be catabolized through the process of autophagy [41]. This diversity of catabolic processes leave open the question of their coordination to resupply the biosynthetic precursors or the energetic compounds and to optimize survival at various timescales.

Optimal metabolic fitness in fluctuating environments requires a time-dependent regulation of storage material, but also of biosynthetic ATP-consuming processes and catabolic ATP-generating processes. While the biosynthetic machinery is switched off in any stress condition, the regulation of the ATP production through the TCA cycle is more subtle and is prone to depend on the nature of the stress. As a result, optimal regulation in various stress conditions tend to require a crosstalk between specialized signaling pathways that have both cooperative or opposing actions on selected enzymatic targets. The obtained pattern of regulation display close similarity with the AMPK and TOR-dependent pathways, as these pathways exert antagonistic roles for storage management, autophagy, and biosynthesis whereas both activate some other pathways such as glycolysis and mitochondrial activity. However, it remains debatable whether regulation should be mediated through post-translational or transcriptional mechanisms, given that transcription-dependent or degradation-dependent changes of expression can be too slow to track environmental changes [38, 42], while rapid protein turnover can be energetically costly. Although the optimal regulatory profile of enzyme exhibited a clear and consistent pattern, the issue of optimal sensing cues remains more difficult to apprehend. An external perturbation is propagated simultaneously through both the metabolic and signaling network in a complex manner as different perturbation modes can be either amplified or attenuated in time. On the one hand, external perturbation seems to provide a more reliable cues. On the other hand, internal sensing provides informations about the metabolic state, that is how well-balanced are the respective flux [16], or how close a system is far from steady state or from the threshold beyond which metabolic collapse occurs. Combined mechanisms of ATP homeostasis and fast ATP turnover make the level of ATP:ADP:AMP ratios very sensitive to whether the metabolic stability is threatened or compromised, and such ratios therefore constitute good indicators of stress [7].

From a single-cell perspective, a primary role of intracellular signaling is to track environmental changes so as to adjust the cellular state accordingly. However, efficient metabolic adaptations in microbial organisms to environmental changes can also occur in the absence of signaling through bet-hedging strategy based on the relative growth and survival rates of cells within multistable and heterogeneous population [43]. In fact, which strategy is optimal and whether these strategies could be mixed depend on many cellular and environmental parameters, such as the rates of proliferation, the randomness and frequency of environmental changes, or the timescale and energetic cost of regulation [44–46], which is reliant on the organism’s lifestyle, prokaryote or eukaryote, unicellular or multicellular, phototroph or chemotroph. The issue of the cellular response strategy to nutrient and energy stress thus provides a promising venue for investigating the evolution of regulatory complexity.

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