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Review

Integrating metabolic pathway fluxes with gene-to-enzyme expression rates

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Abstract

The concept of symmorphosis emphasizes that sequential steps in physiological systems are structurally and functionally matched to each other and to in vivo maximum loads. Examination of metabolic pathways, specifically glycolysis, in this framework led to several conclusions. (i) Linked enzyme catalyzed reaction sequences are so closely integrated with each other that large changes in flux through the pathway are sustained with minimal changes in concentrations of pathway intermediates. This is true for both low and high capacity pathways and is consistent with the 'economic design' expectations of symmorphosis. (ii) In the glycolytic pathway, some enzymes (termed *h*E) occur at high concentrations and high activities, while others (*l*E), usually enzymes operating in vivo far from equilibrium, occur at lower concentrations and lower activities. Although genes for glycolytic enzymes are thought to be coordinately regulated by being linked to common inducing or repressing signals, during long term (phylogenetic) up or down regulation of glycolytic capacity, the expression of genes for *h*E type enzymes are adjusted the most; the expression of *l*E type (usually enzymes functioning far from equilibrium) are up or down regulated the least. These differences are of lower magnitude but are also evident in short term up or down regulation of the pathway of glycolysis (such as induction by hypoxia and repression during electrical stimulation and fiber type transformation in muscle). (iii) When considered together, these data require that, despite coordinate regulation of the overall functional unit (glycolysis), the expression pathway for each enzyme in the sequence must be under unique feedback regulation, implying an unique information flow circuit (gene → enzyme → gene) for each enzyme in the metabolic pathway. (iv) The matching of flux capacities in linked sequences thus seems to apply 'across the board'—not only horizontally but also vertically—in cell metabolic design. That is, up or down change in demand for glycolytic function (horizontal pathway) is integrated with up or down regulation of gene expression (vertical pathway). While a general feedback loop from metabolism to genes has been previously recognized, the step-by-step specificity required for the pathway as a whole has been overlooked. The intriguing question of how enzymes within a single pathway self modulate or fine tune their own expression rate according to their functional role in the pathway remains unanswered, although a number of potential regulatory mechanisms are known.

Keywords: Metabolic regulation; Gene expression; Enzyme expression; Isoform expression; Symmorphosis

Abbreviations: ALD, aldolase; ATP, adenosine triphosphate; ENOL, enolase; EPO, erythropoietin; GLUT, glucose transporter; GPDH, glyceraldehyde-3-phosphate dehydrogenase; *h*E and *l*E, enzymes in a metabolic pathway occurring at relatively high and low concentrations, respectively; HIF1, hypoxia inducible factor 1; LDH, lactate dehydrogenase; mef2, myocyte enhancer factor 2; MRF4, myocyte regulatory factor 4; myf5, myocyte factor 5; MyoD, myoblast differentiation regulatory protein; PDH, pyruvate dehydrogenase complex; PFK, phospofructokinase-1; PGI, phosphoglucoisomerase; PGlM, phosphoglyceromutase; PGK, phosphoglycerate kinase; PGM, phospoglucomutase; PHOS, glycogen phosphorylase; PK, pyruvate kinase; VEGF, vascular endothelial growth factor.

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1. The high precision of metabolic pathway integration

It is axiomatic that many physiological and molecular functions are the sum of individual processes linked in sequence; in isolation many such individual processes have no clear functions at all. How such systems are designed has been of interest to both physiologists and biochemists. For molecular systems at the cellular level of organization, the individual processes are enzyme catalyzed reactions; the linked sequences that give physiological meaning to the component reactions are metabolic pathways. Integrated function can be evaluated by comparing changes in flux through the pathway per se with changes in concentrations of substrates and products of individual enzyme reactions within the pathway. Such approaches very early on indicated that enzymes in multistep pathways are surprisingly well integrated [52,53]. Even in extreme cases, such as in very high capacity metabolic pathways in insect flight muscles [71] or in the electric organ of electric fishes [10,11], several hundred fold flux changes in pathways of ATP demand and ATP supply can be achieved with only minor perturbation in concentrations of pathway intermediates (see abbreviations). To explain this precision and integration of linked sequences of enzyme function, several regulatory models are currently being evaluated by workers in this field [2–4,6,17,26,33,34,42]. These include: (i) Simple feedback and mass action controls [40]; (ii) allosteric controls [3,4]; (iii) models involving the regulation of e_0 (the concentration of functional catalytic sites [34]) by means of alteration in protein interactions (as in actomyosin ATPase [29]), by change in phosphorylation state (as in PDH [50]), by change in redox state (as in V-type ATPases [31]), or by translocation from inactive to an active intracellular location (as in glucose transporters [48]); and (iv) various versions [12,13,23,38,65] of metabolic control analysis first advanced over a decade ago. Whichever of these models best account for the behaviour of any given metabolic system, the empirical observation is that enzymes linked in linear series to form metabolic pathways are so exquisitely integrated that large changes in pathway flux are sustained with minimal perturbation of pathway substrates and products. This is observed over and over again, for low capacity and high capacity pathways.

2. Integrating linked sequences in the whole organism

While these models were being developed in metabolic regulation research, the problem of integrating linked sequences of functions at the whole organism level was being examined for the path of oxygen from lungs to mitochondria by Weibel, Taylor, and their collaborators [62–64,72,73]. In this case, the individual structures are not enzymes; they are the lungs, the circulation, and the working tissues. The function is in vivo oxygen transfer and the pathway is relatively linear but requires inter-organ integration. These workers hypothesized an economically designed pathway wherein, through adjustments in molecular, physiological, or structural parameters, each step in the linked sequence for oxygen transfer was matched to all other steps and to the maximum physiological rate of flux, J_{max} (referred to by others [20] as the natural load of the system). These ideas of economic design became lodged in the literature as the concept of symmorphosis [72]. In their data base (which took advantage of systematic differences between small and large animals and between athletic vs. relatively sedentary species) evidence was found for most steps being well matched to each other and to J_{max}. Only the lungs seemed exceptional, in most species being expressed with excess capacity, i.e. with a larger margin of safety than at other steps in the transfer of oxygen to working tissues. These studies, along with later ones examining the coadaptation of pathways for fuel and for oxygen delivery [73], were heuristic and led to extensive discourse. Personal discussions between one of us (PWH) and both Taylor and Weibel at various times during this period considered the numerous similarities between metabolic regulation and symmorphosis research, but basically no serious comparative studies were done until recently [58–61].

3. Applying symmorphosis concepts to metabolic regulation

Some of the dialogue that followed the Weibel/Taylor work affected metabolic regulation research and led Suarez to three new concepts that were not explicit in this literature prior to the symmorphosis research (at best, they were implicit). The three concepts are related [60,61]. The first is that in a given metabolic pathway, all steps must be matched to the natural load; the maximum velocity (V_{max}) under simulated in vivo conditions for any given enzyme catalyzed step, must be scaled to the J_{max} for the pathway. Secondly, the Weibel/Taylor emphasis on structure in functional integration, when considered at the enzyme pathway level, means maintaining enzyme concentrations at required levels; at each step in the pathway there must be enough but not too much enzyme. Thirdly, implicit in the above and added onto the symmorphosis concept by Diamond, is the idea that at all steps in the pathway there must remain margins of safety or 'reserve capacity' [20].

When trying to evaluate these concepts, the first problem which we must consider is that the catalytic and regulatory properties of enzymes in a given path-

way are not the same. The pathway of glycolysis, for which there are good data available [9,24,59], is composed of some enzymes which are reversible and operate near-equilibrium, while others are allosteric and still others are phorphorylation controlled, often essentially irreversible in vivo. Similarly, the turnover numbers of enzymes in the sequence vary substantially [24]. Since enzymes with such different catalytic and regulatory properties are linked in sequence to form metabolic pathways, it is not surprising that the amount of enzyme required at any step for sustaining in vivo functions may be reaction specific. Probably the two key parameters determining how much enzyme is required at each step for matching in vivo pathway fluxes are: (i) The catalytic efficiency or turnover number of the enzyme (μmol substrate converted to product per μmol enzyme per min); and (ii) the distance from equilibrium at which the enzyme operates in vivo [24,58,59]. These two factors need not be related, but a generalization often found in the literature is that highest turnover numbers are found for equilibrium enzymes. For a given flux in vivo, enzymes with low turnover numbers functioning near equilibrium are at one extreme and by definition are required at relatively high concentrations; for convenience, we shall refer to enzymes within a pathway that occur at high concentrations for a variety of reasons as *h*E. At the other extreme, much lower concentrations of enzymes with high turnover number functioning far from equilibrium are required to sustain a given in vivo flux; we shall refer to enzymes that occur at low concentration relative to *h*E as *l*E. If catalytic efficiency and distance from equilibrium were the only two factors determining the amount of enzyme required, then comparable enzyme concentration profiles for a pathway such as glycolysis would occur in all tissues examined. While similar patterns of *h*E and *l*E enzymes in glycolysis may be seen when comparing homolgous tissues [7,9,24,30,32,57], this is not always observed [24], so other regulatory factors (such as the need for bidirectional or glycolytic vs. glucogenic function) may also be important. For example, along with a regulatory cascade of protein kinases and phosphatases, glycogen phosphorylase is well known to be 'plated out' on glycogen granules and this structural feature may influence the required amount of enzyme [32]. In any event, what we would expect and find [24] is that component enzymes vary in a spectrum from *l*E to *h*E depending upon tissue specific metabolic conditions. Traditionally, metabolic biochemists refer to *h*E enzymes as 'equilibrium enzymes', while *l*E enzymes are 'regulatory enzymes', either allosteric or phosphorylation controlled. It is recognized that there is a gradation between these in terms of in vivo concentration, distance from equilibrium for in vivo function and quantitative contribution to control in varying metabolic states. So-called 'equilibrium' enzymes, such as ENOL, can sometimes operate quite far from equilibrium and display surprisingly high control coefficients [38].

With this background, the questions we wish to address are: (i) During upwards or downwards scaling of the glycolytic capacity of a tissue, how are enzymes with such different properties scaled to match each other and the J_{max} of the pathway? and (ii) are there similarities in pathway design with symmorphosis principles derived for the respiratory system? Two ways to examine these questions are to compare how these different kinds of enzymes are adjusted during large scale upwards or downwards scaling of glycolysis: (i) in evolutionary time; and (ii) in short-term perturbations such as during hypoxia acclimation or during use-disuse (electrical stimulation) transformations.

4. Long term regulation of the glycolytic path

The different kinds of muscle fibers found in vertebrates provide an interesting system for probing these issues. In fish, the myotomal muscles are typically morphologically and spatially differentiated into slow twitch or red muscles (RM) and fast twitch glycolytic white muscles (WM). Qualitatively, the patterns of *h*E and *l*E enzymes in the glycolytic pathway are similar in both muscle types. Through evolution within specific lineages and through ontogeny within species like the tuna, the capacity of the glycolytic pathway in white muscle is drastically up-regulated compared to the pathway in red muscle [30–32]. To achieve this large scale up-regulation, the enzymes for each individual step are changed to variable degree: HK and PFK change the least, while TPI and LDH increase the most. PHOS, PGM, PGI, ALD, PGK, ENOL and PK in tuna WM are elevated by an intermediate amount and are expressed at $\sim$3–5-fold higher levels than in RM. Analyses of low capacity to high capacity glycolytic pathways in different combinations (low capacity RM $\rightarrow$ high capacity RM; low capacity RM $\rightarrow$ high capacity WM; low capacity WM $\rightarrow$ high capacity WM) always show the same result: the largest relative changes are for *h*E type enzymes [32]. Similar conclusions derive from comparisons of mammalian slow and fast muscles: the activities of PHOS, PFK, GPDH, PK, and LDH, in rat quadriceps (mainly fast twitch) muscle exceed those in soleus (mainly slow twitch) muscle by 3–6-fold [7]. In this case, the RM vs. WM relative differences between *h*E and *l*E type of enzymes are not as striking as in fish, probably in part because fiber types are mixed in mammalian muscles.

In these studies, and others discussed below, the actual parameters usually measured are enzyme catalytic activities. However, the isoforms of most of the WM and RM glycolytic enzymes overlap and the turnover number for each enzyme-catalyzed reaction

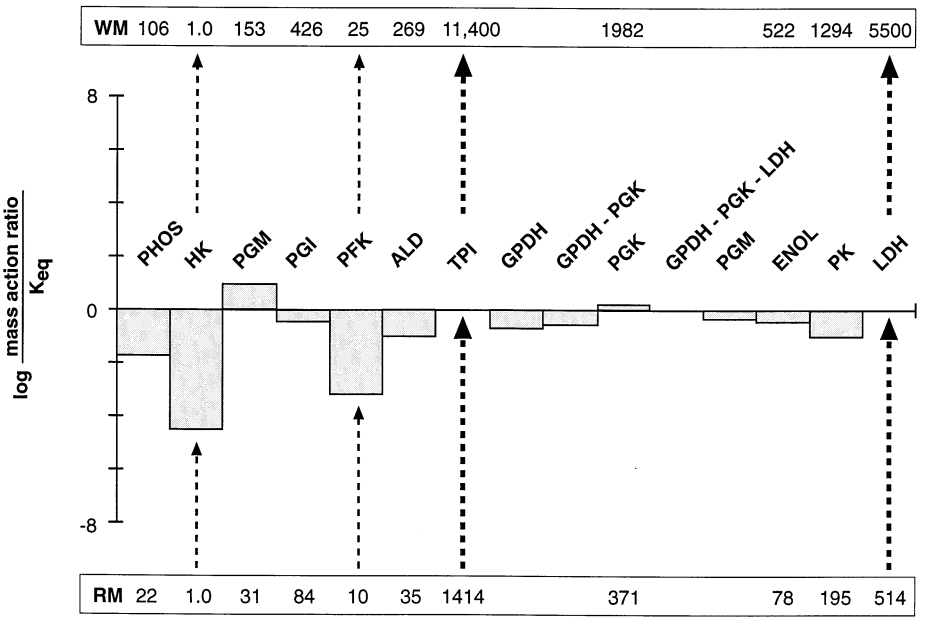

Fig. 1. A summary diagram illustrating the distance from equilibrium (log of the observed mass action ratio/K_{eq}) for glycolytic enzymes in muscle during steady state performance. These particular data were obtained from swimming rainbow trout [21,49], but the pattern is similar for the pathway in most muscles [38], and probably most tissues. Equilibrium enzymes operate relatively close to equilibrium (hence the nomenclature). Regulatory enzymes, such as PFK, operate far from equilibrium. Enzyme activities for the pathway in tuna red muscle (RM) and white muscle (WM) are given below and above, respectively. Enzyme activities (in μmol substrate converted per g tissue per min) are from Guppy et al. [30] and Hochachka [32]. Within each pathway, equilibrium enzymes typically occur at high levels compared to regulatory enzymes, a feature which may be influenced by enzyme turnover numbers. Up regulating glycolytic capacity from RM to WM shows that equilibrium enzymes are increased the most (TPI and LDH shown with dark arrows); regulatory enzymes are increased the least (HK and PFK shown with lighter arrows). These patterns are seen for the glycolytic path in many other biological settings and are observed for other metabolic pathways. See Sections 4 and 5 for further details.

step is similar in the two tissues [24,32]. Since measured maximum activities are linear functions of enzyme concentrations, fish RM vs. WM enzyme activities are probably reasonable reflections of enzyme concentration profiles. Actually, the enzymes of glycolysis are so extensively studied that it is commonly assumed their catalytic activities are a good reflection of enzyme concentration per se; a recent exhaustive analysis [57], for example, shows that ratios of catalytic activities per g of tissue are similar to ratios of molar concentrations of catalytic sites. Because of the *h*E–*l*E structure of the glycolytic path in fish muscles, this means that up regulating overall pathway capacity requires differential (enzyme-specific) amounts of synthesis: more absolute amounts of synthesis of *h*E than of *l*E type enzymes.

Since the turnover numbers for homologous (WM and RM) glycolytic enzymes are similar [24,32], at any given step another determinant of location on the *h*E–*l*E spectrum is the distance from equilibrium for in vivo function [58]. We would thus predict and indeed find a general relationship between the degree of up regulation of different enzymes and the degree of disequilibrium of in vivo function (Fig. 1). To illustrate the situation, the amounts of each enzyme in tuna RM and WM are compared on a plot of each enzymes distance from equilibrium during steady state muscle work [21,49]. The degree of disequilibrium at each enzyme reaction is similar in fish muscles [21,30,32,49] to that observed in the heart [38]; while this pattern may well be common to the glycolytic path in all tissues, fine details vary in different tissues or in different metabolic states [38]. Nevertheless, in the fish muscle example, enzymes such as TPI and LDH seem to function closest to equilibrium and the activities of these are increased the most in the up regulation of RM to WM glycolytic capacity. This is similar to the situation in mammalian muscles and explains why TPI, a quintessential equilibrium enzyme driven by evolution to catalytic perfection (i.e. a state where the turnover number is so high that maximum catalytic velocity is limited by diffusion based enzyme-substrate interaction [24]), nevertheless is required at higher concentrations (approaching the mM range) than any other enzyme in the glycolytic sequence [57]. With in vivo substrate and product mass action ratios almost at thermodynamic equilibrium, TPI at any lower concentrations would be unable to match required fluxes during activated glycolysis.

Regulatory enzymes such as PFK, which under physiological conditions are considered to be largely irreversible, are seen to function far from equilibrium (Fig. 1), as in the heart [38]. The driving force for such enzymes when activated by allosteric positive modula-

tors is high and presumably for this reason, up regulating glycolytic capacities from RM to WM levels requires modest increases in enzyme concentrations. Any remaining short fall in catalytic capacity of such enzymes could be easily compensated for by positive modulators. (The similar HK levels in tuna RM and WM despite much higher glycolytic capacity of the latter probably reflects the observation that WM relies mostly on glycogen, not glucose, as fuel for the pathway [30]). Other enzymes, such as PHOS, PGM, PGI, ALD, GPDH, PGlM, ENOL, and PK, operate at intermediate distances from equilibrium, again as in the heart [38], and intermediate increases in enzyme concentrations occur in WM compared to RM.

Apparently what this means is that through evolutionary time the genetic program for glycolysis has been set so that for a high capacity pathway *h*E type enzymes are up regulated the most, while *l*E enzymes are increased the least. If this same situation applied to short term regulation of the glycolytic path, it would require separate genetic controls for each enzyme in the pathway. The paradox is that in short-term adjustments (such as in hypoxic induction of the glycolytic path and in muscle electrical stimulation where repression of glycolytic enzymes is observed) the genes for the pathway of glycolysis are coordinately regulated as a single unit.

5. Short term regulation of the glycolytic path

Early studies of this problem by Murphy et al. [46] and Robin et al. [51] showed that on exposure to hypoxia, cells in culture increased expression of enzymes in glycolysis, while simultaneously decreasing the expression of enzymes in aerobic metabolic pathways. On average, all glycolytic enzyme expression in L8 myoblasts increased by $\sim$2.4-fold in hypoxia; these are modest changes compared to the long term evolutionary adjustments noted above. Subsequent studies [69,70] showed that the reciprocal coordination (of genes for glycolytic vs. oxidative enzymes) occurs at the transcription level, and more recently still [22,25,37,54,67,68], molecular studies revealed that glycolytic up regulation during hypoxia is preceded by induction of a transcription factor (Hypoxia Inducible Factor 1 or HIF1). HIF1, a basic helix-loop-helix (bHLH) heterodimeric transcription factor [37,68], appears to be part of a universal oxygen sensing and signal transduction pathway involved in the hypoxia dependent regulation of genes for proteins as different as EPO, VEGF1, GLUT1, GLUT3 and glycolytic enzymes, including PFK L, ALD A, GPDH, ENOL1, PGK1 and LDH A [22,25,54]. Indeed, it was the search for hypoxia mediated regulation of EPO expression [16] that led to the discovery of additional roles for HIF1 in control of glycolytic expression. The picture of coordinated hypoxia control of the glycolytic path that is emerging is one of each gene having its own promoter and enhancer sequences (HIF1 binding sites), although not all the glycolytic genes have so far been analyzed.

An important feature of these data, not emphasized by Murphy or Robin [46,51] or anyone else working in the field, is that conditions for hypoxic induction of the pathway are different for *h*E vs. *l*E type enzymes. As in the examples given above for skeletal muscle glycolysis, tissue culture L8 cells in normoxia express large amounts of some enzymes and small amounts of others [51]. In hypoxia, the same percentage induction of an *h*E enzyme such as LDH and an allosteric *l*E enzyme such as PFK requires that quite differing amounts of absolute enzyme product be made in the two cases—a lot of the former, a little of the latter—a theme we have already seen above in comparisons of the glycolytic path in fish red and white muscles. Although this pattern has not been systematically explored, at least some control appears to lie at transcription. In Hela cells for example, normoxic expression rates require a lot more mRNA for LDH than for PFK; furthermore, hypoxia induces a lot more mRNA for LDH than for PFK [22]. A qualitatively similar picture emerges when we analyze the data for glycolytic enzyme activities (i) in transformed cells compared to quiescent cells or in cells stimulated by mitogenic agents to proliferate compared to quiescent cells [19]; and (ii) in electrically stimulated muscles which typically sustain a large induction of enzymes in aerobic metabolic pathways, but a repression of glycolytic enzymes [55]. Parenthetically, we might add that analogous differences betwee *h*E and *l*E enzymes are observed during induction or repression of enzymes in aerobic metabolism [46,51], although the genetic controls here are complicated by participation of nuclear and mitochondrial genes [44,70] and hypoxic repression is not HIF1 mediated [22].

6. Adjusting transcription and translation rates

Taken together, these data uncover two features in the genetic regulation of metabolic pathways like glycolysis. First of all, genes for glycolytic enzymes are coordinately expressed, even if they do not appear to be regulated as operons. In fact, operons in eukaryotic systems have only recently been identified [75] and do not seem to be widespread [28]. Instead, coordinate control is achieved by connecting each gene to common inducing signals (such as hypoxia).

This feature of coordinate control perhaps is to be expected [22,25,37,54,67,68]. The second control feature of the pathway is not; namely, that the flux rate from each gene to each enzyme in the pathway seems to depend on the kind of enzyme being synthesized. High

expression rates, required for *h*E enzymes often operating close to equilibrium, are scaled down to low expression rates for *l*E enzymes usually operating further from equilibrium. To our knowledge, mechanisms are not yet known by which the different genes of the glycolytic sequence can be differentially activated (unique gene → enzyme flux for each enzyme in the pathway). However, there are several control or amplification mechanisms possible at various stages between transcription and completion of protein synthesis. Thus, indications of differential mRNA synthesis for equilibrium vs. regulatory enzymes, observed but not systematically researched [22], suggest at least some gene-specific [27] control at transcription. Recent studies also raise the possibility of control of mRNA stability. A major pathway of mRNA degradation in yeast cells involves the shortening of the poly(A) tail which triggers a decapping reaction and so exposes mRNA to 5′ → 3′ degradation; mRNA-specific rates of decapping and decay could result from differences in interactions between the decapping enzyme and gene-specific transcripts [8]. Finally, it is possible that during hypoxic induction flux rates from genes to enzymes for all steps in glycolysis are the same, and that differing amounts of products are maintained by differential proteolysis. However, this would be wasteful of carbon and energy (a kind of futile cycle or energy short circuit) and would still confront the same specificity problem as above (knowing which enzymes to degrade and at what rate). Besides, despite a large data base on protein synthesis and proteolysis, none suggests that degradation rates vary with the *h*E vs. *l*E nature of the enzyme target [1,36,47].

The problem with the above kinds of control concepts is that they beg the question: how does each gene 'know' the functional (*h*E or *l*E) state of the enzyme it specifies? In other words, how does it 'know' how much enzyme product to make? Analagous control problems in other areas of cell function are often solved by negative feedback modulation by the product of the pathway; in our case, this would require feedback by the protein being expressed. A good example of just such a negative feedback control involves β-tubulin autoregulation of its own synthesis rate by means of an unique amino-terminal tetrapeptide mediating transcript specific-mRNA decay [5]. The α-tubulin isoform also modulates its own synthesis rate through mRNA stability, but the decay pathway for this mRNA is not currently understood.

In summary then we are left with an interesting situation (Fig. 2): either: (i) We assume a feedback loop from enzyme product synthesized to the gene or mRNA being activated, a unique feedback loop for each gene in the glycolytic sequence or (ii) we assume that each gene → enzyme pathway is genetically programmed and uniquely primed for unique rates of transcription, translation, and protein assembly. While both may be intellectually appealing, available studies do not allow clear resolution of the issue at this time.

7. Integrating directional (horizontal and vertical) fluxes simultaneously

Our analysis so far leads to the conclusion that an interesting information flow circuit is required: genes → enzymes → genes. The first arm of this has been generally understood for decades; a special version is the well-known induction of energy yielding pathways by increase in energy demand of working muscles [55,56,66,74]. However, the second arm of the circuit (enzymes → genes), to this point has been under-emphasized and it differs from the kinds of concepts that are pervasive in studies of muscle training and use-disuse transitions. In muscle, this control loop (i.e. feedback from metabolism to genes) is an important mechanism underlying tissue plasticity; it is traditionally considered to be initiated by altered activity or energy demand (use, disuse, denervation, or electrical stimulation) but to be mediated (i) by low molecular weight metabolites (such as creatine, spermidine, or spermine), or (ii) by hormones [66,74]. The possibility of a direct enzyme → gene control loop so far remains unexamined in muscle, but such regulation is known in other systems. In proline metabolism in bacteria, for example, one protein has two activities: proline dehydrogenase and pyrroline-5-carboxylate dehydrogenase which together catalyze oxidation of proline to glutamate. When exogenous proline is available, this complex enzyme is attached to the cell membrane and plays its normal metabolic function. In the absence of proline, it detaches, binds to DNA, and represses expression of both proline dehydrogenase and a proline transport protein, the switch from membrane-bound enzyme to DNA-binding transcription factor being mediated by a change in the redox state of the enzyme [14,45]. Some glycolytic enzymes such as PGK have specific DNA binding sites [15] and this also raises the possibility for direct enzyme → gene control circuitry.

Whatever the mechanism, this feedback, an acknowledged basis of muscle plasticity, shares certain features with muscle differentiation. Current studies indicate that muscle differentiation depends upon four myogenic bHLH transcription factors (MyoD, myogenin, myf5 and MRF4), whose function is potentiated by myocyte enhancer factors, especially MEF2 [43]. The genes for these bHLH transcription factors are referred to as 'master genes' because they in turn regulate batteries of genes further downstream. Interestingly, fiber type specific plasticity is also regulated by bHLH proteins; disuse atrophy, for example, induces MRF4 but not myogenin in slow muscles, whereas myogenin but not

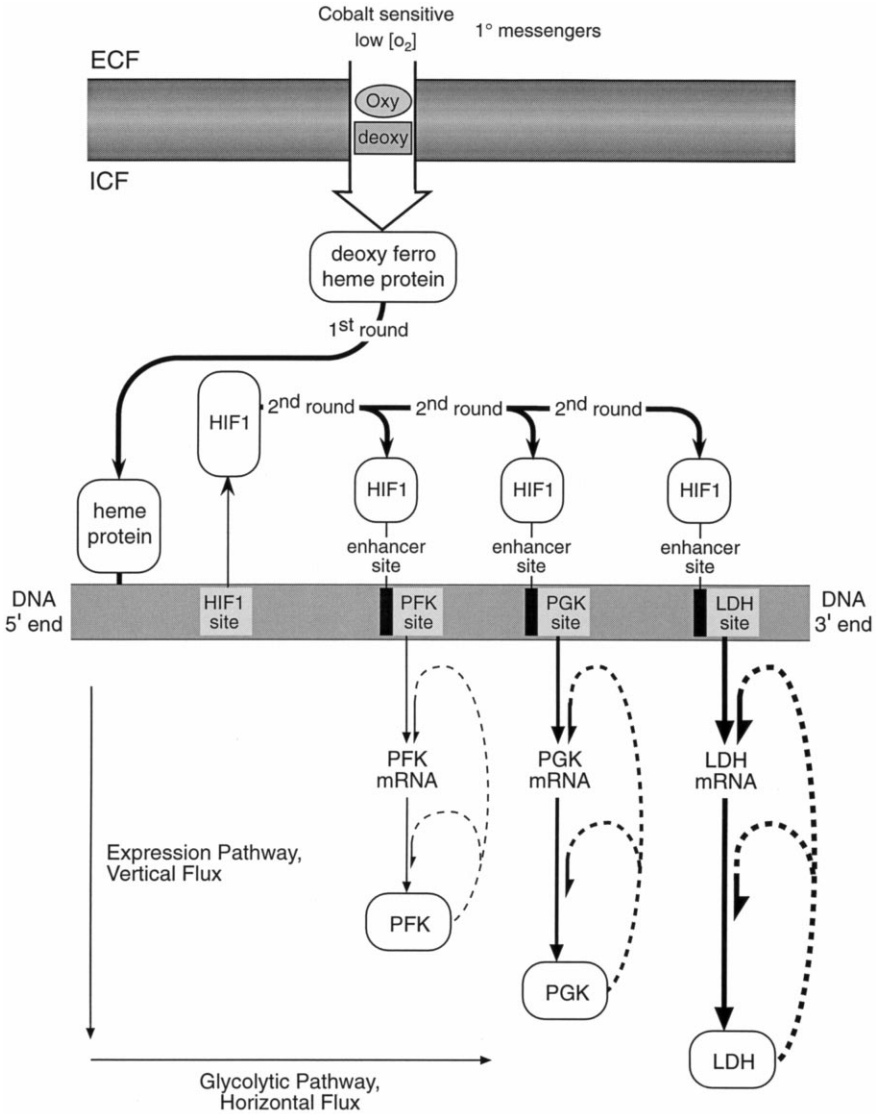

Fig. 2. A summary diagram of hypoxia up regulation of the glycolytic path in hypoxia responsive tissues. A poorly understood oxygen sensing and signal transduction pathway leads to the synthesis of HIF1, hypoxia inducible factor 1 (first round of gene activation). This transcription factor is thought to coordinate hypoxic induction of the pathway: each gene for each glycolytic enzyme maintains a HIF1 binding site (enhancer-mediated second round of expression activation shown for LDH, PGK, and PFK). For any given gene, the enhancer sequence, first discovered in EPO regulation, may be upstream or downstream, within or outside of the promoter sequences. During hypoxic induction of the pathway, each enzyme seems to modulate its own expression pathway (shown as dotted feedback loops; dark arrows indicating large positive modulation, lighter arrows indicating less potent activation). Control mechanisms could act on transcription by interacting with the HIF1 control path [35], at translation through mRNA effects [5,8,18,39]), or post-translationally [74]. Degree of induction (absolute amount of protein synthesis) is greater for *h*E than for *l*E type enzymes. In terms of symmorphosis, vertical expression pathways are fine tuned during hypoxic up regulation to match the new horizontal pathway flux capacity. The differing response patterns of *h*E and *l*E enzymes apply to other- and possibly to all-metabolic pathways. See Section 6 for further details and references. The diagram is based on an analysis of hypoxia defense mechanisms [35] and on HIF1 studies mainly by the Semenza and Ratcliffe laboratories [22,25,37,54,67].

MRF4 transcripts rise dramatically in fast-twitch muscle [41]. It is tempting to assume that similar controls also could contribute to the differential up or down regulation of specific enzymes in metabolic pathways such as glycolysis, but thus far this has not been evaluated.

For molecular physiologists, the significance of the enzyme → gene feedback loop is especially significant for it may mean that the integration of linked enzyme sequences is occurring simultaneously in two directions (Fig. 2): horizontally (flux through the linked enzyme reactions of the glycolytic path) and vertically (flux from genes to enzymes). The former can be a very high flux system (moment to moment response time); the latter of course is a much lower flux system (response time of hours to days) and its functional capacity appears to vary with cellular energy demand. That is why biological processes such as exercise training, elec-

trical stimulation of muscle, or hypoxia exposure typically alter the expression rates of genes for enzymes in glycolysis or in oxidative metabolic pathways. Even if gene → enzyme pathways are low flux and each may be a relatively minor energy drain, when all are combined their cost becomes significant for under normoxic conditions protein synthesis contributes about 1/3 of the maintenance energy demands of the resting cell [35]. If the cost of ATP dependent proteolysis is included, the overall cost of the control circuit (genes → proteins → genes) accounts for ≈50% of normoxic ATP turnover rates [35].

Although it is implicit in the Weibel/Taylor [72,73] studies that the conditions of symmorphosis apply at all (morphological to molecular) levels of organization, to our knowledge this is the first indication of expression pathway (vertical flux) capacities for functionally linked enzymes being optimized or coadapted with enzyme pathway (horizontal) flux capacity (Fig. 2).

Many of the studies in this area to this point have not been designed to specifically address the issues of integration of linked enzyme function and their adaptation; many of them have been more focused on whether genes are on or off in varying metabolic states, rather than whether they are sustaining quantitative changes in expression rates. That may be why the relationships we have uncovered have been largely overlooked.

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References

[1] Argiles JM, Lopez-Soriano FJ. The ubiquitin-dependent proteolytic pathway in skeletal muscle: Its role in pathological states. Trends Pharm Sci 1996;17:223–6.
[2] Arthur PG, Hogan MC, Wagner PD, Hochachka PW. Modelling the effects of hypoxia on ATP turnover in exercising muscle. J Appl Physiol 1992;73:737–60.
[3] Atkinson DE. Cellular Energy Metabolism and its Regulation. New York: Academic Press, 1977.
[4] Atkinson DE. Control of Metabolic Processes. New York:Plenum, 1990: 11–27.
[5] Bachurski CJ, Theodorakis NG, Coulson RMR, Cleveland DW. An amino-terminal tetrapeptide specifies cotranslational degradation of β-tubulin but not α-tubulin mRNAs. Mol Cell Biol 1994;14:4076–86.
[6] Balban RS. Regulation of oxidative phosphorylation in the mammalian cell. Am J Physiol 1990;258:C377–89.
[7] Baldwin KM, Winder WW, Terjung RL, Holloszy JO. Glycolytic enzymes in different types of skeletal muscle: Adaptation to exercise. Am J Physiol 1973;225:962–6.
[8] Beelman CA, Stevens A, Caponigro G, LaGrandeur TE, Hatfield L, Fortner DW, Parker R. An essential component of the decapping enzyme required for normal rates of mRNA turnover. Nature 1996;382:642–6.
[9] Betts DF, Srivastava DK. The rationalization of high enzyme concentrations in metabolic pathways such as glycolysis. J Theor Biol 1991;151:155–67.
[10] Blum H, Balschi JA, Johnson RG Jr. Coupled in vivo activity of the membrane band Na^+ K^+ ATPase in resting and stimulated electric organ of the electric fish *Narcine brasiliensis*. J Biol Chem 1991;266:10254–9.
[11] Blum H, Nioka S, Johnson RG Jr. Activation of the Na^+ K^+ ATPase in *Narcine brasiliensis*. Proc Natl Acad Sci USA 1990;87:1247–51.
[12] Brand MD. Control analysis of energy metabolism in mitochondria. Biochem Soc Trans 1995;23:371–6.
[13] Brand MD. Regulation analysis of energy metabolism. J Exp Biol 1997;200:193–202.
[14] Brown ED, Wood JM. Conformational change and membrane association of the PutA protein are coincident with reduction of its FAD cofactor for proline. J Biol Chem 1993;268:8972–9.
[15] Bryant JA, Ibrhaim A, Brice DC, Fitchet PN, Wang XW, Anderson LE. Does a glycolytic enzyme also participate directly in DNA replication? SEB Abstracts C8 1997;6:107–8.
[16] Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev 1996;76:839–85.
[17] Chance B, Leigh JS Jr, Kent J, McCully K. Metabolic control principles and ^{31}P NMR. Fed Proc 1986;45:2915–20.
[18] Czyzyk-Kreska MF, Dominski Z, Kole R, Millhorn DE. Hypoxia stimulates binding of a cytoplasmic protein to a pyrimidine-rich sequence in the 3′-untranslated region of rat tyrosine hydroxylase mRNA. J Biol Chem 1994;269:9940–5.
[19] Darville MI, Antoine IV, Martens-Stritjhagen JR, Dupriez VI, Rousseau G. An E2F-dependent late-serum-response promoter in a gene that controls glycolysis. Oncogene 1995;11:1509–17.
[20] Diamond J, Hammond K. The matches achieved by natural selection between biological capacities and their natural loads. Experientia 1992;48:551–7.
[21] Dobson GP, Hochachka PW. Role of glycolysis in adenylate depletion and repletion during work and recovery in teleost white muscle. J Exp Biol 1987;129:125–40.
[22] Ebert BL, Gleadle JM, O'Rourke JF, Bartlett SM, Poulton J, Ratcliffe PJ. Isoenzyme-specific regulation of genes involved in energy metabolism by hypoxia: Similarities with the regulation of erythropoietin. Biochem J 1996;313:809–14.
[23] Fell DA, Thomas ST. Physiological control of metabolic flux: The requirements for multisite regulation. Biochem J 1995;311:35–9.
[24] Fersht A. Enzyme Structure and Mechanism. Reading: Freeman, 1985:1–282.
[25] Firth JD, Ebert BL, Ratcliffe PJ. Hypoxic regulation of LDH A: Interaction between hypoxia inducible factor 1 and cAMP response elements. J Biol Chem 1995;270:21021–7.
[26] From AHL, Zimmer SD, Michurski SP, Mohanakrishnan P, Ulstad VK, Thomas WJ, Ugurbil K. Regulation of oxidative phosphorylation in the intact cell. Biochemistry 1990;29:3733–43.
[27] Gallie DR, Feder JN, Schimke RT, Walbot V. Post-transcriptional regulation in higher eukaryotes: The role of the reporter gene in controlling expression. Mol Gen Genet 1991;228:258–64.
[28] Goodridge AG. Dietary regulation of gene expression: Enzymes involved in carbohydrate and lipid metabolism. Annu Rev Nutr 1987;7:157–85.

[29] Grabarek Z, Tao T, Gergely J. Molecular mechanism of troponin-C function. J Muscle Res Cell Motil 1992;13:383–93.
[30] Guppy M, Hulbert WC, Hochachka PW. Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolite profiles. J Exp Biol 1979;82:303–20.
[31] Harvey WR, Wieczorek H. Animal plasma membrane energization by chemiosmotic H + V ATPases control. J Exp Biol 1997;200:203–16.
[32] Hochachka PW. Living Without Oxygen. Cambridge, MA: Harvard University Press, 1980:1–181.
[33] Hochachka PW. Muscles and Molecular and Metabolic Machines. Boca Raton, FL: CRC Press, 1994:1–157.
[34] Hochachka PW, Matheson GO. Regulation of ATP turnover over broad dynamic muscle work ranges. J Appl Physiol 1992;73:570–5.
[35] Hochachka PW, Buck LT, Doll C, Land SC. Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc Natl Acad Sci USA 1996;93:9493–9.
[36] Hochstrasser M. Protein degradation or regulation: Ub the judge. Cell 1996;84:813–5.
[37] Jiang B-H, Rue E, Wang GL, Roe R, Semenza GL. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 1996;271:17771–8.
[38] Kashiwaya Y, Sato K, Tshuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV. Control of glucose utilization in working perfused rat heart. J Biol Chem 1994;269:25502–14.
[39] Kollia P, Fibach E, Najjar SM, Schechter A, Noguchi CT. Modifications of RNA processing modulate the expression of hemoglobin genes. Proc Natl Acad Sci USA 1996;93:5693–8.
[40] Kushmerick MJ, Meyer RA, Brown TR. Regulation of oxygen consumption in fast- and slow-twitch muscle. Am J Physiol 1992;263:C598–606.
[41] Loughna PT, Brownson C. Two myogenic regulatory factor transcripts exhibit muscle-specific responses to disuse and passive stretch in adult rats. FEBS Lett 1996;390:304–6.
[42] McCormack JG, Denton RM. The role of Ca^{2+} transport and matrix Ca in signal transduction in mammalian tissues. Biochim Biophys Acta 1990;1018:287–91.
[43] Molkentin JD, Black BL, Martin JF, Olson EN. Cooperative activation of muscle gene expression by MEF2 and myogenic bHLH proteins. Cell 1995;83:1125–36.
[44] Moyes CC, Mathieu-Costello O, Tsuchya N, Filburn CF, Hansford RG. Mitochondrial biogenesis in myogenesis. Physiologist 1996;39:71.
[45] Muro-Pastor AM, Maloy S. Proline dehydrogenase activity of the transcriptional repressor PutA is required for induction of the put operon by proline. J Biol Chem 1995;270:9819–27.
[46] Murphy BJ, Robin ED, Tapper DP, Wong RJ, Clayton DA. Hypoxic coordinate regulation of mitochondrial enzymes in mammalian cells. Science 1984;223:707–9.
[47] Nair KS, Schwenk WF. Factors controlling muscle protein synthesis and degradation. Curr Opin Neurol 1994;7:471–4.
[48] Norris AJ, Martin SS, Harita T, Nelson JG, Vollenweider P, Gustafson TA, Mueckler M, Rose DW, Olefsky JM. Evidence for an insulin substrate 1 independent insulin signaling pathway that mediates insulin responsive glucose transporter (GLUT4) translocation. Proc Natl Acad Sci USA 1996;93:8401–6.
[49] Parkhouse WS, Dobson GP, Hochachka PW. Control of glycogenolysis in rainbow trout muscle during exercise. Can J Zool 1988;66:345–51.
[50] Patel MS, Harris RA. Mammalian α-keto acid dehydrogenase complexes: Gene regulation and genetic defects. FASEB J 1995;9:1161–72.
[51] Robin ED, Murphy BJ, Theodore J. Coordinate regulation of glycolysisn by hypoxia in mammalian cells. J Cell Physiol 1984;118:287–90.
[52] Rowan AN, Newsholme EA. Changes in the contents of adenine nucleotides and intermediates of glycolysis and the citric acid cycle in flight muscle of the locust upon flight and the relationship to the control of the cycle. Biochem J 1979;178:209–16.
[53] Sacktor B. Biochemical adaptations for flight in the insects. Biochem Soc Symp 1976;41:111–31.
[54] Semenza GL, Roth PH, Fang H-M, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia inducible factor 1. J Biol Chem 1994;269:23757–63.
[55] Simoneau J-A, Pette D. Species-specific effects of chronic stimulation upon tibialis anterior muscle in mouse, rat, guinea pig, and rabbit. Pflug Arch 1988;412:86–92.
[56] Simoneau J-A, Hood DA, Pette D. Species-specific responses in enzyme activities of anaerobic and aerobic energy metabolism to increased contractile activity. Int Series Sports Sci 1990;21:95–104.
[57] Srivastava DK, Bernhard SA. Metabolite transfer via enzyme–enzyme complexes. Science 1986;234:1080–6.
[58] Staples JF, Suarez RK. Honeybee flight muscle phosphoglucoseisomerase: Matching enzyme capacities to flux requirements at a near-equilibrium reaction. J Exp Biol 1997;200:1247–54.
[59] Suarez RK. Hummingbird flight: Sustaining the highest mass-specific metabolic rates among vertebrates. Experientia 1992;48:565–70.
[60] Suarez RK, Lighton JRB, Joos B, Roberts SP, Harrison JF. Energy metabolism, enzymatic flux capacities, and metabolic flux rates in flying honeybees. Proc Natl Acad Sci USA 1996;93:12616–20.
[61] Suarez RK, Staples JF, Lighton JRB, West TG. Relationships between enzymatic flux capacities and metabolic flux rates: nonequilibrium reactions in glycolysis. Proc Natl Acad Sci USA accepted for publication, 1997;94:7065–9.
[62] Taylor CR, Weibel ER, Weber J-M, Vock R, Hoppeler J, Roberts TJ, Brichon G. Design of the oxygen and substrate pathways. I. Model and strategy to test symmorphosis in a network structure. J Exp Biol 1996;199:1643–9.
[63] Taylor CR, Weibel ER, Karas RH, Hoppeler H. Adaptive variation in the mammalian respiratory system in relation to energy demand. Respir Physiol 1987;69:1–127.
[64] Taylor CR, Maloiy GMO, Weibel ER, Langman VA, Kamau JMZ, Seeherman HJ, Heglund NC. Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: Wild and domestic mammals. Respir Physiol 1981;44:25–37.
[65] Thoms S, Fell DA. Design of metabolic control for large flux changes. J Theor Biol 1996;182:285–98.
[66] Viru A. Molecular cellular mechanisms of training effects. J Sports Med Phys Fit 1994;34:309–22.
[67] Wang GL, Semenza GL. J Biol Chem 1995;270:1230–7.
[68] Wang GL, Jiang B-H, Rue EA, Semenza GL. Hypoxia inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular oxygen tension. Proc Natl Acad Sci USA 1995;92:5510–4.
[69] Webster KA, Murphy BJ. Regulation of tissue-specific glycolytic isozyme genes: Coordinate response to oxygen availability in myogenic cells. Can J Zool 1988;66:1046–58.
[70] Webster KA, Gunning P, Hardeman E, Wallace DC, Kedes L. Coordinate reciprocal trends in glycolytic and mitochondrial transcript accumulations during the in vitro differentiation of human myoblasts. J Cell Physiol 1990;142:566–73.
[71] Wegener G, Bolas NM, Thomas AAG. Locust flight metabolism studied in vivo with ^{31}P NMR spectroscopy. J Comp Physiol B 1991;161:247–56.
[72] Weibel ER, Taylor CR, Hoppeler H. The concept of symmorphosis: A testable hypothesis of structure-function relationships. Proc Natl Acad Sci USA 1991;88:10357–61.

[73] Weibel ER, Taylor CR, Weber J-M, Vock R, Roberts TJ, Hoppeler H. Design of the oxygen and substrate pathways. VII. Different structural limits for oxygen and substrates to muscle and mitochondria. J Exp Biol 1996;199:1699–709.
[74] Williams RS, Neufer PD. Regulation of gene expression in skeletal muscle by contractile activity. Handb Physiol Sect 1996;12:1124–50.
[75] Zorio DAR, Cheng NN, Blumenthal T, Spieth J. Operons as a common form of chromosomal organization in *C. elegans*. Nature 1994;372:270–2.

Note added in proof

During review stages, we became aware of studies showing that the relationship between concentrations of mRNA and protein products is fairly plastic. Thus, fasting causes a drop in LDH and in mRNA for LDH, while actin and its mRNA are stable [3]; [LDH] but not [mRNA] increase with body size in barred sand bass [4]; [PK] and [mRNA] increase during growth in trout but this relationship is lost during maturation [1]; and finally, [LDH] and [mRNA] are linearly correlated between northern and southern populations of *Fundulus*, but this relationship is lost durning cold (10°C) acclimation [2].

References

[1] Burness GP, Hochachka PW and Moyes CD. Molecular basis of scaling of glycolytic enzymes in fish. FASEB J 1998; A3944.
[2] Segal JA, Crawford DL, LDH β expression: the mechanism of altered gene expression in acclimation and evolutionary adaptation. Am J Physiol 1994, 267, R1150–3.
[3] Yang TH and Somero GN. Fasting reduces protein and messenger RNA concentrations for lactate dehydrogenase but not for actin in white muscle of scorpion fish (*Scorpanena guttata*, Teleostei) Mol Marine Biol Biotech 1996a, 153–61.
[4] Yang TH and Somero GN. Activity of lactate dehydrogenase but not its concentration of messenger RNA increase with body size in barred sand bass, *Paralabrax nebulifer* (Teleostei) Biol Bull 1996b; 191, 155–8.