Transcriptional profiling provides a powerful means for examining global gene expression patterns for possible teleologic relationships without making a priori assumptions [1]. Unfortunately, decisions regarding which data subsets to report are not always exempt from such considerations. Thus, the selective reporting of transcriptosome information can pose a significant dilemma for author and reader alike. The tendency is to publish only those data reflecting the greatest changes. This practice presumes that the largest differences will be the most significant functionally, and in most instances, this is probably a reasonable assumption. However, thresholds for reporting differences vary widely across datasets, primarily on the basis of statistical considerations that are sometimes arbitrary from a physiologic or metabolic perspective. Changes associated with processes involving multiple, functionally redundant species are particularly problematic and may not be individually robust enough for identification. In such cases, incomplete data reporting may inadvertently result in errors of omission that introduce artificial biases or promote misleading functional inferences. The recent report by Kobayashi et al. [2] illustrates some of these potential pitfalls. These authors describe postphagocytic changes in leukocyte transcript abundance for a number of important metabolic genes that are temporally associated with changes in transcript abundance for genes associated with apoptotic susceptibility. The suggestion that this represents a coordinated gene expression program linking metabolism to apoptosis is both intriguing and plausible. However, the accompanying mechanistic inferences are not fully supported by the data, so a few alternative interpretations are considered below.

The reported increases in post-phagocytic glucose (Glc) disappearance and lactate accumulation are consistent with increased glycolytic metabolism, although the suggestion that decreased Glc phosphorylating capacity, vis-à-vis hexokinase (HK) activity, contributes to these changes is biochemically implausible. By catalyzing the first committed step of Glc metabolism, the phosphorylation of Glc to yield Glc-6-P, HKs are responsible for the initiation of all physiologically relevant pathways of exogenous Glc utilization. Thus, HKs act proximal to all metabolic pathways invoked to explain the authors’ findings. For example, phosphorylation by HKs is a prerequisite for Glc flux through the pentose phosphate pathway (PPP). The oxidative reactions of this pathway constitute the chief cellular source of reduced nicotinamide adenine dinucleotide phosphate for the bioreduction of glutathione, thioredoxin, and biliverdin. Thus, the reported increase in reduced glutathione content provides strong biochemical evidence of increased PPP flux. However, it is unlikely that primary decreases in cellular Glc phosphorylating capacity would lead to increased PPP flux.

Inferences regarding functional coupling between glycolysis and oxidative phosphorylation are similarly problematic, insofar as the coupling reactions are ostensibly dependent on glycolytic flux for substrate provision. These inferences rely largely on transcriptosome information alone. This effectively equates transcript abundance to cognate enzyme function and ignores both the concept of distributive metabolic control and a number of important controlling influences within the cell, including substrate availability, feedback inhibition, and intracellular compartmentalization [3]. Thus, changes in downstream components of individual metabolic flux pathways are insufficient to explain the apparent discrepancy between HK transcript abundance and Glc use. A simpler and more plausible explanation could involve directionally opposite changes in unreported HK isoforms with greater relative contributions to the total, and there is a rational basis for such speculation. Although widely expressed, HKIII does not constitute the principal isoform in any tissue examined [4], including leukocytes, which express several high-affinity HK isoforms [5, 6]. Activities corresponding to HKI and HKIII are well described in these cells, and HKII has been reported in low abundance [6]. However, HKI clearly predominates. Assuming that the microarray is representative of the total expressed leukocyte gene pool, it would be useful to know the magnitude and direction of corresponding changes in HKI and HKII abundance.

The proposed link between glycolysis and apoptosis also warrants scrutiny. The authors suggest that decreased expression of HK and antiapoptotic regulators of HK activity (e.g., phosphatidylinositol 3-kinase and Akt) contribute to the increased apoptosis observed in their model. It is important to note, however, that the antiapoptotic effects of these factors are Glc-dependent [7–9]. The corresponding relationship between Glc and cell death is thus fundamentally different and directionally opposite that described by Kobayashi et al. [2]. The reported proapoptotic effects of Glc in the latter case could suggest specific contributions by increased reactive oxygen species generation [10] or uncoupled cytosolic adenosine 5'-triphosphate (ATP) hydrolysis [11], changes that would be predicted to functionally oppose some of the suggested meta-

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totic effects of HKs have been uniformly associated with the mitochondria-binding isoforms, HKI [7, 9] and HKII [12]. Such salutary effects have not been reported for HKIII, which lacks an amino-terminal mitochondri-al-binding domain. As this isoform is inhibited by physiologic levels of Glc [4, 13], it is also reasonable to assume that decreased HKIII abundance would be functionally apparent at much lower Glc concentrations than those examined by Kobayashi et al. [2].

Granulocytes depend on glycolysis for normal function and can use either exogenous Glc or intracellular glycogen as alternative sources of glycolytic substrate [14–16]. Although free Glc is the preferred substrate under other conditions, a preference for glycogen has been reported following phagocytosis, even in the presence of extracellular Glc [14–16]. Thus, phosphohexoses derived from glycogenolysis could also contribute to the apparent discrepancy between HKIII transcript abundance and downstream metabolism. It is of considerable interest that increases in exogenous Glc use were not accompanied by corresponding changes in cellular ATP content. This could suggest metabolic uncoupling or parallel increases in generation and consumption. Similar findings have been reported in conjunction with increased post-phagocytic glycogenolysis in the same model [14], but the absence of corresponding increases in extracellular Glc utilization [14–16] contrasts markedly with the changes reported by Kobayashi et al. [2]. The apparent stoichiometric relationship between extracellular Glc disappearance and lactate accumulation suggests that glycolysis was the principal metabolic fate of exogenous Glc in this study, although the confounding influence of glycogen-derived lactate cannot be excluded.

In summary, the report by Kobayashi et al. [2] illustrates the considerable limitations and pitfalls of making functional inferences from partial gene expression profiling information. Despite the descriptive power of these types of studies, the ability to glean biologic meaning from them still requires a suitable conceptual framework [17] and appropriate supporting functional data. In this case, the authors have raised a number of intriguing questions for future studies to address, and many investigators would argue that this is where the principal strength of these studies lie. However, for profiling studies that aspire to be more than simply descriptive, a more rigorous, standardized approach may be needed to effectively bridge the enormous gap that presently divides expression- and function-targeted lines of inquiry. Where multiple, functionally redundant isoforms coexist, a compelling case can be made for presenting the corresponding expression data for all relevant species, if not the entire microarray. Profiles of relative transcript abundance provide little or no insight into the relative functional contributions of individual isoforms, so a means of directly relating transcript abundance to cognate protein abundance and/or activity is also needed. Clearly, it would be impractical to demand a functional correlate for every reported transcriptosomal change, but sufficient data to support major functional inferences should constitute the minimal acceptable standard for these types of studies.

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