



Mini Review

Metabolic Regulation of Cell Differentiation

Elizabeth Frayne 

College of General Studies, University of Phoenix, USA
Email: efrayne@verizon.net

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Abstract: Growing research suggests that metabolism itself may play a role in regulating cellular differentiation. This concept stems from three different experimental approaches involving hypoxia, nutrient metabolites, and phosphate analogues, that appear to modulate cellular differentiation by influencing the available cellular energy needed for biosynthetic processes. Possible explanatory mechanisms that could lead to the integration of changes in metabolism with transcriptional regulation include the availability of key metabolites needed for epigenetic changes to occur and/or kinases that can sense the cellular energy charge.

Keywords: cell differentiation, adenylate energy charge, phosphate analogues

1. Introduction

Current research raises the question of whether metabolic activity is a cause or a consequence of cell differentiation [1-2]. Clearly cell differentiation results from cell specialization and the synthesis of specific macromolecules such as proteins, all of which requires energy. Generally, there is an increase in mRNA and concomitant protein synthesis via enhanced transcription, stabilization of mRNA, and/or the translation of stored mRNAs. We know that signaling mechanisms or cues such as growth factors, retinoic acid, and cytoplasmic determinants, as well as asymmetric cell division, play a role in these processes [3]. Also, epigenetic changes are known to be involved in regulating the decision to adopt a cell fate, such as deciding between a stem cell, progenitor, or a mature cell fate [4]. Less clear, however, is the role of metabolism and the energy charge in the cell during cellular differentiation. Increasing evidence suggests that the energy associated with metabolism may be more than merely a requirement for the steps leading to cell differentiation and may instead influence the degree of differentiation as well as specific decisions related to cell fate, function, and/or morphology. Albeit some steps may not require energy per se, but the primary outcome may be determined by the energy state.

2. Cellular energy charge

There are many steps during cell differentiation that do not require cell or tissue specific proteins but rather require more general housekeeping proteins. These steps include changes in cell shape involving cytoskeletal proteins, energy production via the Krebs's cycle and mitochondrial proteins, and basic biosynthetic activity involving RNA polymerases, ribosomal proteins, etc. All these processes must be coordinated with the available energy and indeed appear to respond

to changes in the cellular energy charge (AMP/ATP and ADP/ATP ratios). For instance, platelet cell aggregation appears to respond to changes in the adenylate energy charge (AEC) rather than total ATP levels [5]. Also, biosynthetic enzymes are well known to be regulated by AEC [6]. Adenosine nucleotides are not only participants in such metabolic reactions, but they also act in the allosteric control of numerous key regulatory enzymes in biosynthetic pathways. When the energy charge increases from 0.6 to 1.0, enzymes controlling anabolic pathways are activated such as citrate lyase and phosphoribosyl pyrophosphate (PRPP) synthetase. In mammalian cells, AMP-activated protein kinase (AMPK) senses when the cell's energy charge is low and switches on energy-producing pathways, while switching off energy-consuming pathways to restore cellular ATP levels [7]. This kinase works in part by phosphorylating metabolic enzymes such as, Acetyl-CoA carboxylase to turn off fatty acid synthesis, as well as transcriptional regulators such as SREBPS (sterol regulatory element-binding proteins) to turn off sterol synthesis. It can also turn off protein synthesis by phosphorylating proteins involved in the regulation of mammalian target of rapamycin complex 1 (mTORC1).

3. Hypoxia

Some studies using hypoxic conditions suggest that the cellular energy charge controls more than basic metabolism by influencing the degree of cellular differentiation and/or cell specific specialization. Restricting, the availability of oxygen is a way to influence the AEC via oxidative phosphorylation [8]. An interesting example of this is seen in studies of induced pluripotent stem cells. When somatic cells are genetically re-programmed to become stem cells, cells go from a state of high oxidative phosphorylation to a state of low oxidative phosphorylation and rely instead on glycolysis [2, 9]. Interestingly, this process can be accelerated by restricting the available oxygen, and presumably the energy charge, which then promotes stem cell formation [10].

4. Energy rich metabolites

Another approach to manipulate energy metabolism during differentiation involves the addition of energy rich metabolites to culture media, such as during the study of neural stem cell differentiation [11]. When the neuronal culture medium is supplemented with a mixture composed of branched-chain amino acids, essential amino acids, TCA cycle precursors and co-factors, the number and characteristic features of neuronal dendrites is increased. The nutrient rich conditions drive mitochondrial remodeling with a resultant increase in cellular ATP that leads to the activation of mTORC1 dependent anabolism. Furthermore, the addition of fatty acids to induced pluripotent cardiomyocytes helps to promote their differentiation into more mature cardiomyocytes [12]. Note, cardiomyocytes are well known to prefer fatty acids as substrates for cellular energy.

5. Phosphate analogues

More recently my own research has found that use of the phosphate analogue, thio-phosphate, during cell culture appears to result in an increase in the cellular energy charge [1]. This analogue reduces RNA turnover by creating phosphorothioate linkages in RNA that inhibit cellular nucleases, which then allows the accumulation of significant amounts of mRNA and other non-ribosomal RNA in the cell. The energy savings from reduced RNA turnover results in an enhanced biosynthetic profile, the result of transcriptional changes rather than differential mRNA stabilization. In *E coli*, RNA seq studies of the analogue show enhancements in the transcription of genes for RNA processing enzymes, ribosomal proteins, tRNA charging enzymes, translational proteins, as well as outer membrane, plasma membrane, and periplasmic proteins. There are also increases in transcripts for genes involved in the synthesis of amino acids, fatty acids, carbohydrates, cell structures, cofactors, vitamins, and secondary metabolites. None of these changes correlate mRNA stability, which suggests that transcriptional control mechanisms are at play, in response to the presumed increase in cellular energy. Additional evidence comes from changes in cells observed at the protein level, such as in yeast where thio-phosphate enhances total protein secretion and in HEK293 cells where it appears to enhance the expression of neurofilaments [13].

In *E. coli* dramatic morphological changes are also observed using thio-phosphate instead of phosphate in culture media [1]. Namely, the bacteria form extensive filaments. Prolonged aggregation of flocculant yeast has also been observed (unpublished observations). In the case of Brewer's yeast, the stabilizing impact on mRNA levels may be the cause of this temporary effect, that then changes with continued growth. However, in *E. coli* it appears the presumed increased energy charge results in a new altered morphological state. This suggests that an increase in the cellular energy charge can promote cell specific states. It will be interesting to examine the impact of thio-phosphate in models of cell differentiation in eukaryotes.

6. Possible mechanisms

If the energy charge in the cell does play a role in cell differentiation and/or cell specification, this raises the question of how this might occur. Both eukaryotes and bacteria methylate DNA but bacteria unlike eukaryotes methylate adenine residues in DNA rather than cytosine [14]. Nevertheless, such epigenetic changes could be a possible mechanism. In eukaryotic cells mitochondria may be an important intermediary in this process. It has been suggested that the availability of mitochondrial metabolites such as acetyl-CoA, citrate, and alpha-ketoglutarate involved in histone acetylation and methylation, may impact epigenetic processes that could then modulate gene expression and stem cell decisions [2, 9].

Another likely mechanism by which the energy could regulate transcription is via the phosphorylation of regulatory proteins. While phosphorylation of ser, thr, and try amino acids in bacteria is rare, it does occur [15]. In addition, recent studies indicate that 10% of his residues in the *E. coli* proteome are phosphorylated [16]. So, there could be another kinase much like AMPK that is able to sense the energy charge and respond accordingly. Phosphorylation of sigma factors could certainly result in transcriptional changes. In fact, recent studies using *Vibrio parahaemolyticus* indicate transcriptional regulation via phosphorylation of inactive sigma factors enables them to interact with the beta' subunit of RNA polymerase [17].

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