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What makes the cell differentiate?

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ABSTRACT

In the present paper, I propose a hypothesis whereby the necessity to maintain the permanent energy-dissipating metabolic flux represents the primary force that determines the eukaryotic cell's choice to grow, divide and/or differentiate. This view is based on the universal structure and the strict redox neutrality of the core metabolic network. I propose that the direct substrate level coupling between metabolism and gene expression through epigenetic mechanisms provides a mechanistic explanation of how this control is implemented.

The fundamental question of how cell fate decisions are made gains increasing interest in the context of systemic thinking and latest advances of stem cell biology. According to the prevailing view cell fate decision is considered from a deterministic perspective: as a coordinated change in gene expression patterns in response to external signals. However, strictly deterministic models of cell differentiation are contradicted by experimental evidence (for review and criticism of determinism see (Kupiec, 2009)). To overcome the contradiction and reconcile the idea of deterministic mechanisms with the observed high variability of the differentiation processes, the concept of plasticity is usually introduced in the explanatory scheme. A popular emerging idea is to represent the different phenotypic states of the cells as attractors of a dynamic complex system built up by gene networks and to consider the attractor state as determined by an associated gene expression pattern (Balazsi et al., 2011; Huang et al., 2005). Cell fate change in this framework is thought as the movement from one attractor to another. It is increasingly popular to illustrate the attractor concept using a 3D surface of the hypothetical parameter space of the system reminiscent to the "epigenetic landscape" metaphor initially proposed by Conrad Waddington half a century ago. In this visual metaphor the cell is represented as a ball rolling downhill along the valleys through the epigenetic landscape. The fate decision of the cell is seen as a choice of the ball between two bifurcating valleys. According to this representation the topography of the landscape, in other terms, the possible phenotypic trajectories are determined by the genes, but the fate decision of an individual cell is only indirectly dependent on them; in addition to the topography it is influenced by the previous trajectory (history) of the cell and the local perturbations of the movement.

In the modern version of the epigenetic landscape metaphor valleys are replaced by attractors in the state space of gene networks and are defined by a particular gene expression pattern. Stochastic fluctuations of gene expression are considered as perturbations that are able to induce transition between the attractors (Balazsi et al., 2011; Huang et al., 2005). Although both the original and the modern versions of the landscape metaphor are very useful for the visual illustrations of the epigenetic vision of cell differentiation, the most fundamental question has never been asked: what forces make the cell move downhill through the series of fate decisions? If the metaphor were to be taken seriously the answer would obviously be: gravitation! But what could be the biological equivalent of the "gravitation"? Although very important, regulation of gene expression can hardly be the driving force. The regulatory mechanisms, prokaryotic as well as eukaryotic, are products of a long evolution. The living cells were able to proliferate and presumably differentiate to some extent even before these mechanisms appeared. This raises the question: what fundamental principles orchestrated these basic processes? Are these principles still recognizable in present day living organisms?

Perhaps we can get closer to the answer if we recall that a living cell is an open thermodynamic system far from the equilibrium that constantly dissipates energy to maintain the steady-state. Substantial decrease or disruption of the energy flux leads to death because the structure and function of the cell can not be maintained anymore. This is so essential for life that the basic organization of the energy-producing central carbon metabolism is universal, suggesting that it was already present in the ancestors of

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all present day organisms (Smith and Morowitz, 2004). This universality also suggests that some fundamental principles governing the cell's choice between proliferation and differentiation may be hidden in the architecture of the metabolic network.

Obviously, the requirement of continuous energy dissipation limits the cell's choice to the options that are compatible with the energy production from the available substrates. I put the emphasis here on the "available substrates" because this is an absolute condition for the activation of a metabolic pathway, because no chemical reaction is possible without substrates. Then, the choice between proliferation and differentiation will depend on which option can better ensure the continuous energy flux essential for the cell's survival. The decision is directly conditioned by the substrate availability; regulation can only reinforce and modulate it. Therefore, instead of conferring the central role to sophisticated regulatory and signalling mechanisms and considering the activation of a given metabolic regime as a consequence, the hypothesis proposed here uses reverse reasoning. Inspired by earlier ideas of metabolic and redox signalling (Blackstone, 2000, 2006), I propose that the change in substrate concentration in the cellular environment directly alters the relative contribution of different metabolic pathways to the flux of matter and energy in the cell. The resulting fluctuations in the intracellular concentration of key metabolites, if sufficiently important, trigger a cellular response that includes phenotypic change, i.e. change in gene expression patterns. The key questions then are: (i) what are the differences between the metabolic regime of proliferating and differentiating cells and (ii) how these differences lead to changes in gene expression.

On the basis of our half-century-old textbook knowledge on cellular metabolism we know that the proliferating and differentiating cells use different substrates and different metabolic pathways. Cell division requires strong biosynthetic activity to produce new cell components. By contrast, differentiation and differentiated functions are highly energy-dissipating, but less dependent on biosynthesis. Chemical energy and the building blocks for biosynthesis are generated concomitantly with the flow of electrons from the donor nutrient molecules to oxygen or other electron acceptors through glycolysis/pentose phosphate pathway (PPP), Krebs-cycle and terminal oxidation. These are perhaps the most ancient metabolic pathways; they are universal in all living organism on earth. Roughly speaking, nutrients are transformed into glucose to enter the glycolysis. A single glucose molecule is broken down into two molecules of pyruvic acid through the glycolysis and/or PPP and produces two molecules of ATP with the concomitant transfer of electrons to electron carriers that generates two molecules of NADH (or NADPH in PPP). One more NADH molecule is produced if pyruvate is decarboxylated into acetyl-CoA. Acetyl-CoA represents a crucial branching point (Fig. 1). When oxygen is available, acetyl-CoA enters the Krebs-cycle and oxidized ultimately into carbon dioxide and water. The energy released during the redox reactions is used to generate ATP with the final count of 36 molecules from a single glucose molecule. This is the most efficient way the cell can produce large amount of ATP that is essential for various functions such as the maintenance of membrane potential or contractile protein function. However, carbon dioxide and water are not substrates for the biosynthetic pathways that produce polysaccharides, lipids and polypeptides (except photosynthesis, but this is not in the scope of the present discussion). Biosynthetic pathways use acetyl-CoA as a substrate, or in some cases another intermediates directly related to it (phosphoenol pyruvate, oxaloacetate, and 2-oxoglutarate). Nucleotides for DNA and RNA synthesis are derived from PAP intermediates. In addition, the key steps of the biosynthetic pathways use the reduced electron carriers NADH or NADPH as a source of energy. Therefore, the

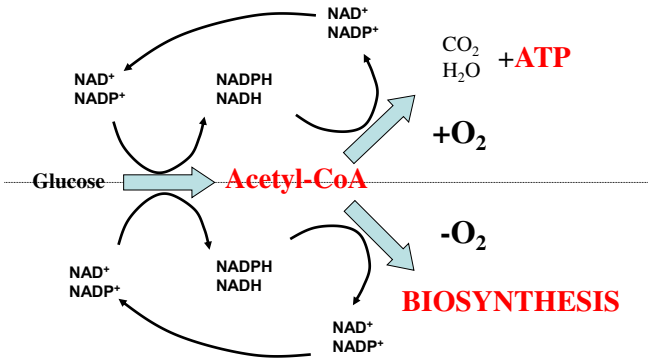

Fig. 1. Schematic representation of the key role of Acetyl-CoA in the central carbon metabolism at the crossroad of the ATP-producing catabolic and biosynthetic pathways.

higher the part of the glucose (nutrients) oxidized by oxygen to yield CO_2, H_2O and ATP, lower is the cell's capacity to synthesise new macromolecules. Therefore, high oxygen/nutrient ratio hampers cell proliferation. On the other hand, this pathway provides the cell with a large amount of energy in the form of ATP and will enable it to perform specialized tasks, as contraction, maintenance of membrane electric potentials etc., beyond the cell's own needs. This provides basis for differentiation and opens the possibility to the "division of labour" between the cells and to the development of multicellular organisms. Another important point is that oxygen is not an ordinary chemical substrate; it is the best possible physiological electron acceptor. The standard redox potential of the $1/2O_2 + 2H + 2e^- \rightarrow H_2O$ pair is the highest among the possible redox pairs. This has an important corollary. In the presence of oxygen the electron flow will be spontaneously directed to it and the reaction chain will have the propensity to ultimately oxidize all carbon to carbon dioxide and reduce oxygen to water leaving no substrates for biosynthesis. Therefore, high oxygen/nutrient ratio increases the cell's tendency to slow down growth and proliferation and orient the cell toward a high ATP-demanding differentiated phenotype. If the electron flux is blocked for any reason, they are likely to escape and react directly with oxygen to form partially reduced reactive oxygen species (ROS) that represent a threat for the cell. Differentiation appears in this context as a self-defence mechanism. If the available carbon resources are not sufficient to reduce the oxygen to H_2O, the cell may even degrade its own components to defend itself from the oxidative stress. This process can enter in an irreversible phase leading to cell death.

However, if the oxygen/nutrient ratio is low there is not sufficient oxygen to oxidize all the nutrients to CO_2 and H_2O. In order to maintain the metabolic flux essential for the cell, the excess glycolysis end products have to be removed and, because the metabolism needs to be redox neutral, the excess NADH and NADPH has to be oxidized. A rapid way to do this is to reduce the pyruvate into lactic acid. In the presence of sufficient amount of nutrients this solution will provide the cell with ATP, it will eliminate the end product of the glycolisis and regenerate the oxidized NAD required for the functioning of the reaction chain. Many protists and cells in multicellurar organisms respond rapidly to hypoxia in this way. On the long run however, this is a loosing strategy, because, in addition to the poor energy balance of this procedure, it results in the rapid acidification of the cell's environment. Fortunately, the excess of pyruvate or of acetyl-CoA derived from pyruvate can be removed by incorporating it into macromolecules by biosynthesis. As mentioned above, essentially all biosynthetic pathways use acetyl-CoA or related intermediates

as starting substrates and the key steps of these pathways use NADH or NADPH as a source of energy. Although at a number of reaction steps ATP is also required, biosynthesis is essentially dependent on the availability of reduced electron carriers. Biosynthesis regenerates the oxidized pool of the electron carriers required for the redox neutrality of the metabolism. Therefore, the newly synthesised molecules play *de facto* the role of the terminal electron acceptor and biosynthesis can be considered as a way to maintain the continuous energy flux. Since the high rate of biosynthesis results in the increase of the biomass, cells in low oxygen/high nutrient environment have the tendency to proliferate at a high rate.

In low oxygen/low nutrient environment the cell encounters serious difficulties. Not only the resources are scarce, the cell's capacity to efficiently extract chemical energy by oxidizing the substrates into water and carbon dioxide is limited as well. Such cells can not proliferate or perform ATP-demanding tasks. At best they enter a quiescent state until the life conditions improve. Since they are not exposed to the damaging effect of ROS, they can remain quiescent for a long period. Inactive undifferentiated stem cells could exemplify this state.

When the oxygen/nutrient ratio is intermediate – probably most of the time – the relative contribution of the catabolic and anabolic pathways to maintain the continuous flux is continuously adjusted by the cell to the changing oxygen/nutrient ratio. Even small changes induce rapid fluctuations in the corresponding fluxes. However, if the alterations in the substrate concentrations are substantial and long-lasting, the cell may need to express new genes to adapt the cell's response to the new environment. The change in metabolic fluxes can be translated into change in gene expression by a direct mechanistic relation. The enzymes that catalyse post-translational epigenetic modifications of the chromatin components use key metabolites as substrates: acetyl-CoA, NAD+, ATP etc. Since these molecules are produced at the crossroads of the major metabolic pathways, rapid and substantial fluctuations of their concentrations are expected when the metabolic flow is modified. Fluctuation of the substrate concentration can directly impact the pattern of epigenetic modifications and confers a direct chemical sensitivity and dependence to the chromatin on the metabolism without the need of a dedicated signalling mechanism. Postulated some time ago (Kupiec, 1996; Paldi, 2003), the link between the metabolism and chromatin stability now well documented (for review see (Katada et al., 2012)). Chromatin opening is the first and essential step for expression of new genes or stable repression of previously expressed ones. The sensibility of chromatin stability to the metabolic flux provides a mechanistic explanation of how the change in gene expression pattern and the resulting phenotypic responses are initiated.

The necessity to maintain the permanent energy-dissipating metabolic flux and the universal structure of the metabolic network can explain why the nutrient/oxygen ratio controls the cell's choice to grow, divide and/or differentiate. Our increasing understanding of the direct substrate level coupling between metabolism and gene expression through epigenetic mechanisms provides a mechanistic explanation of how this control is implemented. This explanatory scheme also provides a rational basis to explain the formation of tissues and ordered multicellularity as a result of the metabolic cooperation and complementation of the cells. If true, this view can also explain how the perturbations of the nutrient/oxygen ration disturb the normal metabolic cooperation and can result in pathologies characterized by the disruption of the normal tissular structure, such as degenerative disorders or cancer. Although most of the existing data are fully consistent with the view described in this paper, dedicated empirical investigations will reveal the exact role of the metabolic control on cell proliferation and differentiation.

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