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Metabolic pathway compartmentalization: an underappreciated opportunity?

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For eukaryotic cells to function properly, they divide their intracellular space in subcellular compartments, each harboring specific metabolic activities. In recent years, it has become increasingly clear that compartmentalization of metabolic pathways is a prerequisite for certain cellular functions. This has for instance been documented for cellular migration, which relies on subcellular localization of glycolysis or mitochondrial respiration in a cell type-dependent manner. Although exciting, this field is still in its infancy, partly due to the limited availability of methods to study the directionality of metabolic pathways and to visualize metabolic processes in distinct cellular compartments. Nonetheless, advances in this field may offer opportunities for innovative strategies to target deregulated compartmentalized metabolism in disease.

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Introduction

Given the complexity of biological systems in multicellular organisms, evolution has organized living matter into structured compartments. At the macroscopic level, the human body is divided in distinct organs and tissues. Organs are composed of multiple cell types, cells are divided into organelles, and organelles in turn are defined by spatial architecture. At the molecular level, cellular metabolism is a prime example of an organized complex system. Scientific reductionism has lead to increasingly better descriptions of cellular metabolic networks [1]. However, cells are not simple bags of enzymes and metabolites with random spatial and temporal organization [2]. On the contrary, metabolic pathways are compartmentalized in time and space to support specific cellular processes. The realization that metabolism controls cell function in health and disease has lead to a resurgent interest in cellular metabolism, with the ultimate goal of targeting metabolism therapeutically. However, it is clear that a better understanding of how metabolic compartmentalization contributes to cell function could help to identify novel, perhaps even more specific, therapeutic approaches. We will not provide an all-encompassing literature survey, but rather discuss representative examples that highlight the importance of metabolic compartmentalization to support cell function.

How and why is metabolism compartmentalized?

Physical delimitation of metabolic pathways within membrane-delineated organelles or the cytosol is a classical example of metabolic compartmentalization [3]. Metabolic pathways can be localized in distinct domains, even within one compartment. For instance, in smooth muscle cells, glycogen stores are metabolized via oxidative phosphorylation, while external glucose is used for glycolysis [4]. Metabolic enzymes can be also sequestered to scaffold proteins, as demonstrated by the association of glycolytic enzymes with the actin cytoskeleton [5,6••,7–9]. Another feature of metabolic enzymes is their ability to assemble in large quaternary structures, ranging from homodimers (fatty acid synthase) and polymers (glutamine synthetase) to multiprotein metabolon complexes (purinosome) [10–13]. Spatial organization of enzyme complexes facilitates transfer of metabolites from one enzyme to the next, thus generating an efficient assembly line and increasing overall metabolic output [5,14,15]. In addition to spatial compartmentalization, metabolic pathways are also temporally compartmentalized. A prime example is the transient peak in glycolysis and glutaminolysis during the S phase of the cell cycle [16].

There are many benefits to subcellular compartmentalization. First, subcellular compartmentalized microenvironments favor optimal activity of specific enzymes. For instance, peroxisomal oxidase and catalase activity are higher at alkaline pH [17], as is the case in peroxisomes [18,19]. Second, some metabolic reactions produce toxic intermediates that can be contained and disposed of in closed compartments. The containment of hydrogen peroxide and its breakdown by catalase inside peroxisomes is a prime example [20]. Third, compartmentalization can limit diffusion and prevent escape of intermediates

through organelle membranes as is the case for ATP and ADP in muscle cells, which therefore need adenine nucleotide translocase to cross the inner mitochondrial membrane [21]. Fourth, compartmentalization counteracts futile cycles of metabolites that might otherwise be continuously interconverted in reactions operating in opposite directions. In yeast, differential expression of metabolic enzymes periodically occurs over time. Such a precise orchestration regulates energy production through activation of distinct metabolic pathways in restricted temporal windows [22]. Finally, metabolic compartmentalization often increases the efficiency of chemical reactions, as many reactions in free solution would be otherwise diffusion-limited due to restricted local enzyme substrate availability. In brain and muscle, glycogen and glycogenolytic enzymes are compartmentalized together in microdomains to facilitate glycogen breakdown [23]. In the smooth muscle cell example, the internal glycogen stores that are metabolized via oxidative phosphorylation supply energy for actin–myosin contractions, while the external glucose supplies energy for ion transport via glycolysis [24]. Despite the many benefits and obvious necessity of metabolic compartmentalization, its functional role in supporting biological processes is underappreciated. In the remainder of this review, we will provide examples of how metabolic compartmentalization controls specific cell functions.

Meeting energy demand by metabolic pathway compartmentation

An example illustrating how metabolic pathways influence biological processes is the hydra, a primitive animal in which distinct metabolic pathways are selectively preferred depending on the type of locomotion adopted. The slow movement of its body over the sea floor relies on oxidative metabolism, while glycolysis is required for the sudden and dynamic changing movements of its tentacles for praying [25]. Likewise, an association of glycolytic enzymes with structures for rapid motility has been described in the tail of sperm cells [26]. Because glycolysis has the capacity for quick ATP production relative to oxidative phosphorylation, it is used for activities requiring high, local levels of ATP over a short period, whereas oxidative phosphorylation more efficiently produces ATP over time.

Compartmentalization of glycolysis during cell motility

Cellular migration relies on dynamic remodeling of the cytoskeleton, a process requiring large amounts of local ATP [27,28]. Interaction of glycolytic enzymes with the actin cytoskeleton has been documented in mammalian neurons and skeletal muscle cells as well as in fruitflies and fish muscles. Compartmentalization of glycolysis on the actin cytoskeleton enables efficient and high local ATP production [5,7–9]. The importance of this association is illustrated by the fact that, despite expression of the full complement of active glycolytic enzymes in flight muscles, transgenic fruitflies are unable to fly when glycolytic enzymes fail to colocalize in actin-rich sarcomeres [9].

Migrating endothelial cells rely on highly dynamic actin architectures at the leading edge, lamellipodia and filopodia, which pull the cell body forward [29]. In quiescent endothelial cells, mitochondria and glycolytic enzymes are present in the perinuclear cytosol [6••]. In contrast, when these cells start to migrate, they relocate their glycolytic machinery to the lamellipodia and filopodia (Figure 1) [6••]. Notably, glycolytic enzymes physically interact and coconcentrate with actin fibers at the membrane ruffles in the lamellipodia, and even become superactivated by this actin association [6••]. Interestingly, endothelial cell lamellipodia and filopodia are too narrow and too thin to accommodate bulky mitochondria [6••]. Local glycolysis allows rapid generation of ATP for dynamic motility, and prevents taxing ATP demands throughout the cell [6••]. Suppressing glycolysis reduces endothelial cell motility, and lamellipodia and filopodia formation, whereas blocking mitochondrial respiration does not affect endothelial cell migration [6••].

Similar to ECs, cancer cells often have a high glycolytic flux, which has been implicated in cancer cell invasion. Invasive cancer cells migrate through extracellular matrix using protrusive structures called invadopodia [30]. Proteomic analysis of invadopodia revealed that they are enriched in glycolytic enzymes [31]. High glycolytic flux has accordingly been associated with mesenchymal cancer cell motility and cytoskeleton remodeling, possibly accounting for a more aggressive phenotype [84]. Furthermore, reducing glycolysis in cancer cells decreases their ability to degrade the matrix [31,32].

Mitochondrial compartmentalization during cell motility

Mitochondria are also important for migration of various cancer cells, though this requirement is dependent on the cancer cell type. For instance, invadopodia in human glioma contain active mitochondria [33]. In migrating epithelial cancer cells, mitochondria reside at the leading front, and altering their anterior position impairs migration [34]. In metastatic breast cancer cells, mitochondrial fragmentation promotes, while mitochondrial fusion reduces lamellipodia formation and cell migration [35•]. Consistently, inhibition of mitochondrial ATP production reduces migration and actin polymerization, implicating mitochondria as the source of ATP for actin remodeling [35•].

Lymphocytes exhibit a profoundly different mode of motility compared to endothelial and cancer cells. In order to migrate in a directional manner, they undergo polarization and generate specialized cell domains. They form a lamellipodium at the leading edge, containing the actin polymerization machinery and chemoattractant receptors, and a uropod at the trailing edge, concentrating adhesion receptors and the microtubule-organizing center (MTOC)