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Glycolytic Coupling to Mitochondrial Energy Production Ensures Survival in an Oxygen Rich Environment

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The mitochondrion exhibits biochemical and functional variations that emerged by random chance as an evolutionary survival strategy, which include enhanced energy production driven by anaerobic respiratory mechanisms. In invertebrates, this mitochondrial anaerobic respiration permits survival at a lower energy state suited for this type of environment while yielding more ATP than by glycolysis alone. This ability provides a protective existential advantage in naturally occurring hypoxic environments via diminished free radical generation. In the blue mussel *Mytilus edulis* and other marine organisms, a functionally active mitochondrial anaerobic respiratory mechanism tailored to hypoxic conditions reflects an evolutionary adaptation/reworking of ancient metabolic pathways. Components of these pathways were also discovered and characterized as metabolic intermediates in plant parasites, specifically crown gall tumors. Mechanistic similarities between anaerobically functioning mitochondria in *M. edulis* and crown gall tissues and metabolic processes in human tumors are known to occur, demonstrating commonalities in diverse life energy processes. Furthermore, cytoplasmic glycolytic processes are now shown also to exhibit a dynamic capacity for enhanced energy generation by increasing its efficiency in hypoxic environments, making it equally dynamic in meeting its cellular survival goal.

MeSH Keywords: Bacteria • Cell Hypoxia • Glycolysis • Metabolome • Mitochondria • Mitochondrial Diseases

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Why Mytilus? Anaerobic Respiration

The intertidal environment of the marine mussel, *Mytilus edulis,* poses unique metabolic challenges to survival. At low tide, the mussel must close its valves and provide a limited amount of energy under hypoxic conditions, switching to anaerobic respiration [1–3] as do other animals [4,5] and numerous other eukaryotes [6], plants [7,8] and of course, in prokaryotes. This appears to be a well conserved evolutionary strategy utilizing the dynamic coupling capabilities of the mitochondrion and cytoplasm to facilitate metabolic processes dependent on anaerobic respiration [3,6,9]. This strongly suggests these mussels represent an ideal model system to examine these phenomena for their biomedical significance [10–13].

The dynamic nature of this system can be observed in the ability of each tissue type in the mussel to respond differently to hypoxia as a result of mitochondrial functional differences in gene expression, see [1]. This phenomenon itself offers a tremendous opportunity to examine these diverse energy production processes in a single organism, suggesting an environment whereby abnormalities can emerge and be tolerated, e.g., cancer. The gills, digestive glands, mantle, and adductor muscle respond to hypoxia by switching to anaerobic respiration [9,14–17]. The gill with its ciliated epithelium and neural innervations is heavily populated with mitochondria [18,19]. Clearly, this necessitates greater energy requirements, which may be difficult at intertidal intervals. We surmise this difficulty is overcome by way of nervous system integration of the tissue, exerting specific and rapid responses to respiratory and waste needs carried out by the ciliated epithelium in coordination with existing energy generating variations [20,21].

Enhancement of Cytoplasmic Energy Efficiency

The human brain uses about 20% of its oxygen intake in order to meet the high energy demands associated with its functions, e.g., cellular interactions, communications, etc. Clearly abnormal demands on this process could and would compromise mitochondrial function, initially observed in cognitive impairments [10,12,22–25]. Colón-Ramos and others [26] surmised and demonstrated the presence of an "emergency energy mechanism" in the neural tissues of an invertebrate (*Caenorhabditis elegans*). This mechanism appears to emerge in the cytoplasmic domain when much greater than normal energy is required by, in this instance, neural tissues. As previously noted, mitochondria can move under this condition in a cell to respond to hypoxic local regions/events (see [1]). In addition, it can be surmised, energy demands may exceed the mitochondria's abilities under conditions of high demand, thus, causing another energy producing phenomenon to emerge or reemerge within the scope of retained evolution-based molecular history. The older low-energy evolutionary glycolytic system, which still conduits products for energy metabolism, assists in meeting this extra demand by concentrating its efforts anatomically, providing for an enhanced micro-regional cellular energy demand. In so doing, an anatomical and physiological clustering of energy processes occurs in the cytoplasm, demonstrating a hypoxic reflex to low oxygen levels. Colón-Ramos and colleagues refer to this as "glycolytic metabolons"[26]. Thus, cytoplasmic components of this primordial energy system bring into play cytoplasmic processes in an organized and highly localized manner to complement mitochondrial anatomical and physiological insufficiencies. This cellular cytoplasmic energy responsiveness, we surmise, may also be subject to malfunctions, potentially initiating and/or maintaining low oxygen level associated pathologies, e.g., Alzheimer's, Type 2 Diabetes, etc.

We surmise the "metabolon" phenomenon represents a similar process to what occurs in *M. edulis* tissues under hypoxic conditions, e.g., relying on a primordial energy system (glycolytic metabolism) for a supplementary energy supply under adverse hypoxic conditions. New evolutionary processes, aerobic respiration, may exhibit an upper limit for tolerating high free radical generation/presence as also noted by the ability of nitric oxide to modulate mitochondrial complexes [27–29]. Furthermore, the switch between initiating or terminating the use of this older evolutionarily-conserved system may represent a critical event in allowing tissues to sustain unlimited growth, e.g., cancer. In other words, the "switch" in and out of this survival mechanism may be faulty since it relies on free radical levels as regulatory processes, e.g., nitric oxide [1,10,29]. Therefore, this process exists and works because of its evolutionary significance in preserving survival processes, e.g., anti-biosenescent phenomena, via reverting to an early successful phenotype.

It is equally important to note that as eukaryotic cellular evolution progressed it did so from prokaryotes and archaea, incorporating and combining similar energy processes in different organisms by way of stereoselectivity based on a hydrogen core pump system, which provided the needed chemical commonality to preserve a complex communication and regulatory system [30,31]. Early cells, e.g., prokaryotes, evolved and existed under hypoxic/anoxic conditions, demonstrating that these cytoplasmic energy processes probably contained numerous protective and energy generating variations that in all likely hood were maintained in evolution and reemerge today under similar microenvironment conditions. Hence, abnormal energy formation and associated biomedical processes may simply represent previous metabolic strategies for survival, emerging again when conditions occur that represent the "primitive" environment in which they evolved. Thus, extensive cytoplasmic

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processes can come into play when low oxygen levels stimulate their emergence [10].

Further illustrating this hypothesis is the random emergence of photosynthesis and its oxygen byproduct. Organisms that could detoxify increasing levels of oxygen in the environment and yield a H based proton pump associated with enhanced energy output were favored since components of this system were already in place in prokaryotes (nicotinamide adenine dinucleotide). Thus, detoxification via oxygen gave great significance to the presence of oxygen reductases and associated hydrogen pumps, yielding energy production and toxic removal in one seamless process [32]. In this regard, the metagenomic analysis of *Candidatus Lokiarchaeon* is on the top of the list and it is hydrogen dependent, which is essential in claiming mitochondrial ancestry with cytoplasmic coupling [30,33]. Also, since oxygen may have served as the catalyst for the bacterial endosymbiotic relationship, we speculate that if eukaryotic cells were again introduced into a low level oxygen environment, existing mitochondria containing cells would gradually decrease since they would be detrimental.

Translational Importance

Given the subtle nature of these new and old processes, with the commonality of stereospecificity [34,35], finding an initiation event in pathological eukaryotic disorders that may be associated with these phenomena becomes almost impossible since they are so closely coupled and interwoven [10]. For example, at what level and for how long would exposure to low oxygen concentrations lead to vascular perturbations, triggering brain vascular inflammation, leading to Alzheimer's Disorder [36]? In this scenario, genetic polymorphisms/variations would play a role in determining personalized susceptibilities to a common abnormal microenvironment. In short, alleviating early symptoms of the associated disorders may be difficult because of the lack of realization of a common initiating phenomena and event, which has long since passed unnoticed.

As noted earlier, functional similarities between anaerobic mitochondrial subtypes in *M. edulis* and crown gall tissue and metabolic processes in human tumors exists [1,11]. For example, investigators suggest that carcinogenic processes might target normal mitochondrial functioning and cause a disruption of the Krebs cycle and electron transport enzymes [37–40]. In this regard, normative mitochondrial function in non-proliferating cells affects relatively high cytosolic ATP/ADP ratios, resulting in functional inhibition of aerobic glycolysis [41]. In contrast, the bioenergetics of the "Warburg" effect that has been extensively linked to the metabolic phenotype of numerous cancer cell types is characterized by enhanced aerobic glycolysis and suppression of aerobic mitochondrial metabolism [37–40].

In this regard, we hypothesize that enhanced aerobic glycolysis, which may be part of the triggering system in initiating cancerous phenotypes, is a major initiation mechanism. Additionally, aerobic respiration in proliferating cells leads to deleterious production of free radicals that can damage DNA and proteins. Accordingly, free radical damage is proposed to exacerbate compromised mitochondrial functioning thereby diminishing the existential viability of cancer cells [11–13,25]. Along these lines, Davila and Zamarano [42] posit that cancer can be viewed as a cell that has phenotypically reverted to the last common eukaryotic ancestor of the host cell. They surmise that a cancer cell is functioning as a facultative anaerobic microbe with unlimited replication potential, which we surmise is attributable to enhanced glycolytic associated processes since cancer-like cells don't tolerate oxygen well. Interestingly, anaerobic mitochondria in gill cilia of *M. edulis* have evolved to utilize the phenotype of a facultative anaerobe [9,43]. In summary, we hypothesize that switching to a "back-up" or an existing conserved phenotypic energy producing system may occur even when uncalled for and when the microenvironment is similar to previous low-oxygen level environments. If this is the case, we further surmise that it may remain on after it has achieved its demand, thus resulting in various types of cancer, which may be determined by the phenotype expressed in the specific normal cell where and when the event occurs. The fact that this "metabalon" process normally exists, supports our hypothesis. Moreover, higher free radical levels in eukaryotic cells along with genetic expression variations, e.g., polymorphisms, and mitochondrial heteroplasmy create a formidable environment for cellular longevity and survival. Potential dangers, however, are counteracted by constantly emerging cellular and molecular processes functionally linked to enhanced longevity and quality of life.

Conclusions • Explaining the Unexplainable

In speculating about the relationship of mitochondria (bacterial origin) and the eukaryotic cell, we note that this intimate association confers an excellent strategy to survive an environment that was increasingly becoming toxic, e.g., oxygen, while simultaneously offering an abundance of chemical energy (glucose). Thus, by one organism in this relationship dealing with the "fire" and its containment and the other providing fuel for the fire, which would now "warm" both cells, a coping strategy emerged. In this process, the toxic waste would combine with oxygen yielding it harmless and recyclable. In time, the nature of this relationship became even more sophisticated, driven by the high energy capacity, so that its existence would be assured and maintained. Ultimately, it reinforced and provided the energy background for mass DNA replication furthering diverse functions and the foundation for longevity. At the higher energy levels provided by aerobic respiration, DNA

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replication was enhanced and increased in a protective intranuclear environment, that also allowed for positive mutations to emerge quicker. Furthermore, the eukaryotic cell provides one genome and the endosymbiont provides numerous genomes whose influence emerges as a single determinant by their expressed ratio, e.g., heteroplasmy homeostasis. Importantly, their communication and mutual influence occurs primarily at complex one of the electron transport system situated in the mitochondrion at the heart of the "fire". Thus, any malfunction in this relationship would exert profound effects on processes dependent on them, e.g., cellular, tissue, organ, and finally organismic, health. Indeed, critical dysfunctionality would express itself acutely, whereas moderate and low dysfunction would express chronically. In the nature of the changes that could occur during "life", it would also be difficult to define the originating negative event due to anti-biosenescent processes, e.g., migrating mitochondria, hypoxia, heteroplasmy ratios, etc. Hence, the functional and anatomical conservation of enhanced glycolytic processes further demonstrates that during evolution, old "information" can have tremendous functionality for present, and yet to be determined, processes. In this regard, naturally occurring compounds have been and will continue to be found in nature, exhibiting enhanced medicinal properties via glycolytic and aerobic respiration processes. These biogenic molecules will enter into a line of therapeutic agents that will extend longevity and healthier living because of their evolutionary association with regulatory targets. Equally true, their efficacies will be enhanced because of modern biomedical technology, including rapid design to accommodate personalized medicine. In conclusion, the diversity of existing life already contains the information required to live healthy for longer periods of time.

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