Evolution of Cytochrome c Oxidase IV Regulation in Mammals

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ABSTRACT: Aerobic respiration, although metabolically advantageous in O₂-rich environments, can be detrimental to the cell when O₂ is not fully reduced resulting in cytotoxic reactive oxygen species (ROS) production. Cytochrome c oxidase subunit 4 (COX-4) is primarily responsible for fully reducing O₂ during metabolism and exists as COX4-1 and COX4-2 isoforms. The former exists in normoxia, but is replaced by the latter in hypoxia. This change is brought about by two mechanisms, the first involving regulation by hypoxia inducible factor 1 (HIF-1), which directly upregulates COX4-2 and indirectly degrades COX4-1. The second mechanism involves an oxygen responsive element (ORE), which upregulates COX4-2 in a HIF-1 independent manner. The convergence of two unrelated pathways to regulate COX4-1 and COX4-2 would allow cells to optimize their metabolic profile within an environment experiencing varying O₂, such as Earth's early atmosphere in the case of primitive aerobic bacteria or in multicellular organisms where O₂ levels vary between tissues such as lung tissue.

KEYWORDS: COX4, HIF1-alpha, metabolic profile, hypoxia regulation, convergent evolution

This essay is part of a continuing series of student class work completed in a second-year biology course at The University of Western Ontario, introduced [here](#). The essay illustrates one or more principles of evolution on a topic of the student's own choice.

If you observe where eyes are positioned on both Nile crocodiles and hippopotamuses, you will notice that they are situated on the tops of the animals' heads to allow periscope-like vision while the animals' bodies are submerged (Figure 1). Surprisingly, hippopotamuses and crocodiles do not share a recent common ancestor in evolutionary history. Nonetheless, they have adapted to a similar environment the—Nile River—to gain the common competitive advantage of semi-submerged concealment. This example illustrates convergent evolution where unrelated populations evolve to obtain similar functions that increase their fitness in a specific environment. Another example of convergent evolution is evident in the regulation of subunit four of cytochrome c oxidase (COX4) in mammals.

COX4 catalyzes the concerted reduction of O₂ to H₂O ($4e^- + 4H^+ + O_2 \rightarrow 2H_2O$), which maintains the electrochemical gradient across the inner mitochondrial membrane, thereby providing the proton motive force required by ATP synthase to make energy in the form of adenosine triphosphate (ATP). Aerobic respiration uses O₂ to indirectly make ATP and increases ATP production tenfold when compared with anaerobic respiration (1). With the dawn of oxygenic photosynthesis, O₂ levels in Earth's atmosphere increased, thereby giving those individuals that obtained the ability to undergo

aerobic respiration an advantage over those that did not (2). However, O₂ can be toxic to the cell if it is not fully reduced to H₂O; partially reduced forms called reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), damage DNA, proteins, and other cellular molecules (3). The production of ROS increases in hypoxic (low O₂) conditions and as such, those individuals that can safely reduce O₂ are more favoured in environments with varying O₂ levels.

Figure 1. The anatomical positioning of eyes on a crocodile (left) and a hippopotamus (right) allows each of them to remain submerged in their aquatic environments. This illustrates convergent evolution where two evolutionarily unrelated species gain similar functions when placed in a similar environment. Images retrieved from pixabay.com and are public domain under Creative Commons CC0.

To account for these conditions, COX4 exists as two isoforms, aptly named COX4-1 and COX4-2 (4). COX4-1 is better suited to normal O₂ conditions, whereas COX4-2 functions most

efficiently under hypoxic conditions (5). The relative amounts of each protein at a given time are based on their gene expression. These genes are transcribed into messenger RNA (mRNA) in varying amounts depending on cellular conditions or environmental signals. Proteins can interact with promoter sequences in the cell's DNA either to enhance gene expression by attracting transcription proteins to assemble mRNA from DNA or to repress gene expression by preventing recruitment of such machinery. Thus, these regulatory proteins are a means to control mRNA production and moreover protein synthesis.

O₂ regulates the production of COX4-1 and COX4-2 in mammalian cells via regulatory proteins using two different mechanisms. One mechanism involves hypoxia-inducing factor one (HIF1) as the main regulator of both COX4-1 and COX4-2. A dimer of HIF1 α and HIF1 β creates the functional HIF1 protein. In normal O₂ conditions, prolyl hydroxylase hydroxylates HIF1 α at specific proline residues, which are recognized by the von Hippel-Lindau (VHL) protein for binding, ubiquitination, and subsequent degradation by proteasomes, thereby inhibiting HIF1 formation (6) (Figure 2). Under hypoxic conditions however, prolyl hydroxylase is inhibited, which prevents VHL-mediated HIF1 α degradation. HIF1 α is now able to dimerize with HIF1 β to form HIF1, which binds to a DNA sequence within the COX4-2 promoter known as the hypoxia responsive element (HRE). HIF1 increases the production of many proteins including COX4-2 and mitochondrial LON protease (5). LON degrades COX4-1, which is subsequently replaced by COX4-2 (7) (Figure 3). Because COX4-2 works more efficiently in hypoxia than does COX4-1, the cell is able to change its metabolic profile to maximize its energy output while minimizing ROS production. These results led to a hypothesis whereby COX4 isoforms interchange like a HIF1-dependent tag-team (5).

Although some components of COX4 regulation have been elucidated in detail, the discovery of a different pathway to regulate the same protein has suggested that unrelated cellular pathways in early aerobic populations converged to regulate COX4. Early aerobes to optimize their metabolic profile in an O₂-rich environment. This different pathway includes a novel oxygen-responsive element (ORE) comprising a 24-nucleotide sequence downstream of the previously described HRE within the COX4-2 promoter (8). Sequence conservation of the ORE between mammalian tissues suggests that the sequence may

have been present in an ancestor of modern mammals (8). Furthermore, the ORE differs from the HRE with respect to its position and composition in the COX4-2 gene, thus illustrating its lack of homology (ancestral relationship) with the HIF1 pathway (8). Furthermore, gene activity increased more than threefold when an ORE was present under hypoxic conditions, whereas HREs showed negligible activity under identical conditions (8). Conversely, when the ORE is mutated, COX4-2 gene activity plummets, thereby outlining its importance in the O₂-dependent regulatory pathway of COX4-2 (8). Several transcription factors are associated with the ORE. Recombination signal sequence-binding protein Jk (RBPJ) and coiled-coil-helix-coiled-coil-helix domain two (CHCHD2) have been suggested to act in concert to activate COX4-2 in hypoxia, whereas CXXC-type zinc finger protein five (CXXC5) negatively regulated the ORE and downregulated COX4-2 expression in normoxia (9) (Figure 2 and Figure 3). These data further support a HIF1-independent mechanism of COX4-2 regulation, thus displaying how independent molecular pathways can directly regulate the same gene.

It may seem redundant and counterintuitive for a cell to use two different mechanisms to regulate COX4 expression in hypoxia. On the contrary, HIF1 and ORE mediated regulation may have converged over many generations to complement each other, ultimately maximizing cellular respiration during hypoxia. For instance, HIF1 is maximally expressed in complete absence of O₂, yet minimally expressed in hypoxia; the opposite holds true for ORE activity (8,9). A competitive advantage may have been gained by individuals who could account for slight, but potentially detrimental, changes in atmospheric O₂. Another advantage of having two independent mechanisms for COX4 expression is akin to a computer's backup drive; if one mechanism fails, the other will most likely be functional due to its molecular independence, therefore continuing to regulate the expression/repression of the two isoforms.

Furthermore, it has been suggested that these regulatory processes may be tissue-specific depending on the tissue concentrations of O₂. Lung tissue, for instance, would be exposed to greater changes in O₂ and therefore has a higher potential for ROS production than liver tissue (8,9). If this holds true, it may not have been possible for multicellular life to designate one pathway or another in differentiated cells based on their O₂ conditions. Independent molecular pathways that converge on and regulate a common gene (i.e. HRE- and ORE-

regulation of COX4-2) provide such an advantage to differentiated multicellular life.

Figure 2. In presence of O₂, hypoxia-inducible factor 1α (HIF1α) is hydroxylated by prolyl hydroxylase (not shown). von Hippel-Lindau (VHL) protein binds HIF1α and leads to its ubiquitination and subsequent degraded by proteasomes. This leaves the Hypoxia Responses Element (HRE) unbound and prevents COX4-2 expression. CXXC-type zinc finger protein 5 (CXXC5) has also been shown to downregulate COX4-2 in normoxia by binding to the Oxygen Responsive Element (ORE) downstream of the HRE in the COX4-2 promoter (3–9).

Convergent evolution is a powerful tool for sculpting populations as they adapt to their current environments. The function of both HIF- and ORE-mediated regulation is controlled by expression/repression of COX4-1 and COX4-2, which has evidence to support its role in aerobic and moreover mammalian evolution. These independent sequences converged to regulate the same gene to adapt to a hypoxic environment just as the eye positioning of crocodiles and hippopotami converged to a similar anatomical position to adapt to their environment. Convergent evolution can occur on both a macroscopic and molecular level. It is important, however, to remember that evolution is not a means to obtain the perfect species. A species thrives or perishes based on its adaptability to a given environment and evolution is the force that drives the most competitive species forward. Gaining insight to these processes at the molecular level provides us with the knowledge to make connections and further understand the natural world.

Figure 3. In hypoxia, prolyl hydroxylase is inhibited and HIF1α is not hydroxylated (not shown). HIF1α dimerizes with HIF1β forming HIF1 which binds the HRE to upregulate COX4-2. Similarly, recombination signal sequence-binding protein Jk (RBPJ) and coiled-coil-helix-coiled-coil-helix domain 2 (CHCHD2) upregulate COX4-2 expression by directly binding the ORE downstream of the HRE. HIF1 also upregulates LON (a mitochondrial protease) which degrades COX4-1. COX4-2, being more efficient in hypoxia, integrates into the inner mitochondrial membrane to replace COX4-1 and aid in the electron transport chain (not shown) (3–9).

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