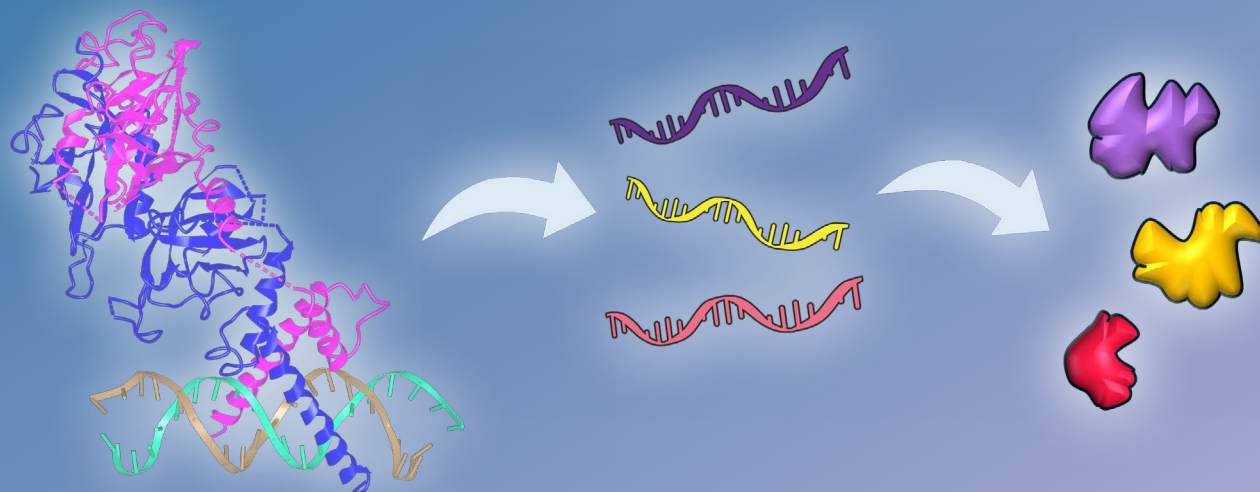




Technical Note

Cellular Transcriptome and Proteome Dynamics Under Hypoxia



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Introduction

Proteome-wide expression changes induced by hypoxia, mediated primarily through hypoxia-inducible factor 1 (HIF-1), represent a cornerstone of cellular adaptation to low oxygen levels. HIF-1, a transcription factor composed of HIF-1 α and HIF-1 β subunits, becomes active during hypoxic conditions when HIF-1 α is stabilized due to reduced hydroxylation by prolyl hydroxylase domain (PHD) enzymes. This stabilization allows HIF-1 α to accumulate, translocate to the nucleus, and initiate transcription of genes containing hypoxia response elements (HREs). These genes encode a broad array of proteins involved in angiogenesis, metabolism, apoptosis regulation, and more.

Several studies have identified HIF-1 and HIF-2 binding sites across the genome under hypoxic conditions (1% oxygen or less) in various cancer cell lines and primary cell types such as human vascular endothelial cells (HUVECs). Depending on the assay used and the cell type, the results suggest a range from 500 to over 2000 unique binding sites for HIF proteins, with more binding sites available under hypoxic conditions than at normoxic (atmospheric 21% oxygen) conditions. This translates into upwards of 1000 genes with altered expression under hypoxia. Taking this to the next level regarding proteins, one study on glioblastoma LN18 cells identified 426 proteins having an altered abundance, while another study identified 340 proteins significantly upregulated and 42 significantly decreased in hypoxic T cells. These data conclude that hypoxic conditions can induce mass transcriptomic and proteomic changes across different cell types, suggesting that the status of cells at normoxic conditions are not the same as those at physiologic or low oxygen.

Metabolic Proteins

Hypoxia induces significant shifts in metabolic proteins. HIF-1 activates the expression of enzymes favoring glycolysis, such as aldolase, hexokinase, phosphoglycerate kinase (PGK) and lactate dehydrogenase A (LDHA), while suppressing mitochondrial respiration by promoting pyruvate dehydrogenase kinase (PDK1). This reprogramming supports ATP production under oxygen-limited conditions. Furthermore, glucose transporter 1 (GLUT1) and monocarboxylate transporter 4 (MCT4) are upregulated, enhancing glucose uptake and lactate export, respectively.

Angiogenesis

Angiogenic proteins are another critical category impacted by hypoxia. HIF-1 directly increases vascular endothelial growth factor (VEGF) levels, promoting new blood vessel formation to restore oxygen supply to hypoxic tissues. Concurrently, matrix metalloproteinases (MMPs) are upregulated to remodel the extracellular matrix, facilitating angiogenesis and cell migration, while the upregulation of RhoA regulates cytoskeletal reorganization for cell migration.

Stress Response

Proteomic studies reveal that hypoxia impacts protein folding and stress response pathways. Chaperones such as heat shock proteins (HSPs) and protein disulfide isomerase (PDI) are upregulated, reflecting an increased demand for protein stabilization and folding in the endoplasmic reticulum. Hypoxia-induced oxidative stress further amplifies the expression of antioxidant proteins like thioredoxin and glutathione-related enzymes, which maintain redox balance critical for cell survival.

Apoptosis/Survival

The role of HIF-1 in apoptosis is complex and depends on the cell type and other factors, potentially leading to pro- or anti-apoptotic paths. HIF-1 upregulates genes like galectin-3 and gelsolin, involved in apoptotic pathways. HIF-1 can increase the expression of BNIP3 and NIX, which inhibit the anti-apoptotic effect of Bcl-2. Additionally, HIF-1 can stabilize wild-type p53, which then activates genes like p21 that cause cell death. In reducing apoptosis, HIF-1 can upregulate Bcl-2 and Bcl-xL, while downregulating Bax, Bak, and Caspase-9.

Cancer

In cancer and other pathological conditions, hypoxia-induced proteomic changes often exacerbate disease progression. For example, HIF-1 activation promotes cancer cell survival, proliferation, and metastasis. Proteins such as β 3 integrins and cadherins are differentially expressed under hypoxia, facilitating invasion and adhesion. These findings highlight the dual role of hypoxia in normal physiological adaptation and disease pathogenesis.

The Hidden Transcriptome of Non-Coding RNAs

In addition to altering gene expression that leads to protein changes, activation of HIF proteins also directly influences RNA molecules, such as microRNAs (miRNA) and long non-coding RNAs (lncRNA), that do not encode for proteins but have functional activity on other molecules. Both miRNAs and lncRNAs, in turn, influence the expression and function of HIF proteins, as well as HIF cofactors. Well over 50 specific mRNAs and lncRNAs have been identified as either up-regulators of HIF and the HIF pathway or down-regulators of HIF. Furthermore, non-coding RNAs can impact the expression of a number of other proteins not directly regulated by HIF.

Epigenetics of Hypoxia

Hypoxia and the HIF-1 pathway exert significant epigenetic effects that modify gene expression to facilitate cellular adaptation to low oxygen levels. Under hypoxic conditions, HIF-1 activates enzymes like histone demethylases (e.g., JMJD1A and JMJD2B), which alter histone methylation patterns, promoting the transcription of hypoxia-responsive genes. Additionally, hypoxia can reduce the activity of histone deacetylases (HDACs), leading to chromatin remodeling and enhanced gene accessibility. DNA methylation changes, mediated by DNA methyltransferases (DNMTs), also occur in hypoxic environments, silencing or activating specific genes involved in angiogenesis, metabolism, and cell survival. Together, these epigenetic modifications form a dynamic regulatory framework, allowing cells to fine-tune gene and protein expression in response to oxygen deprivation.

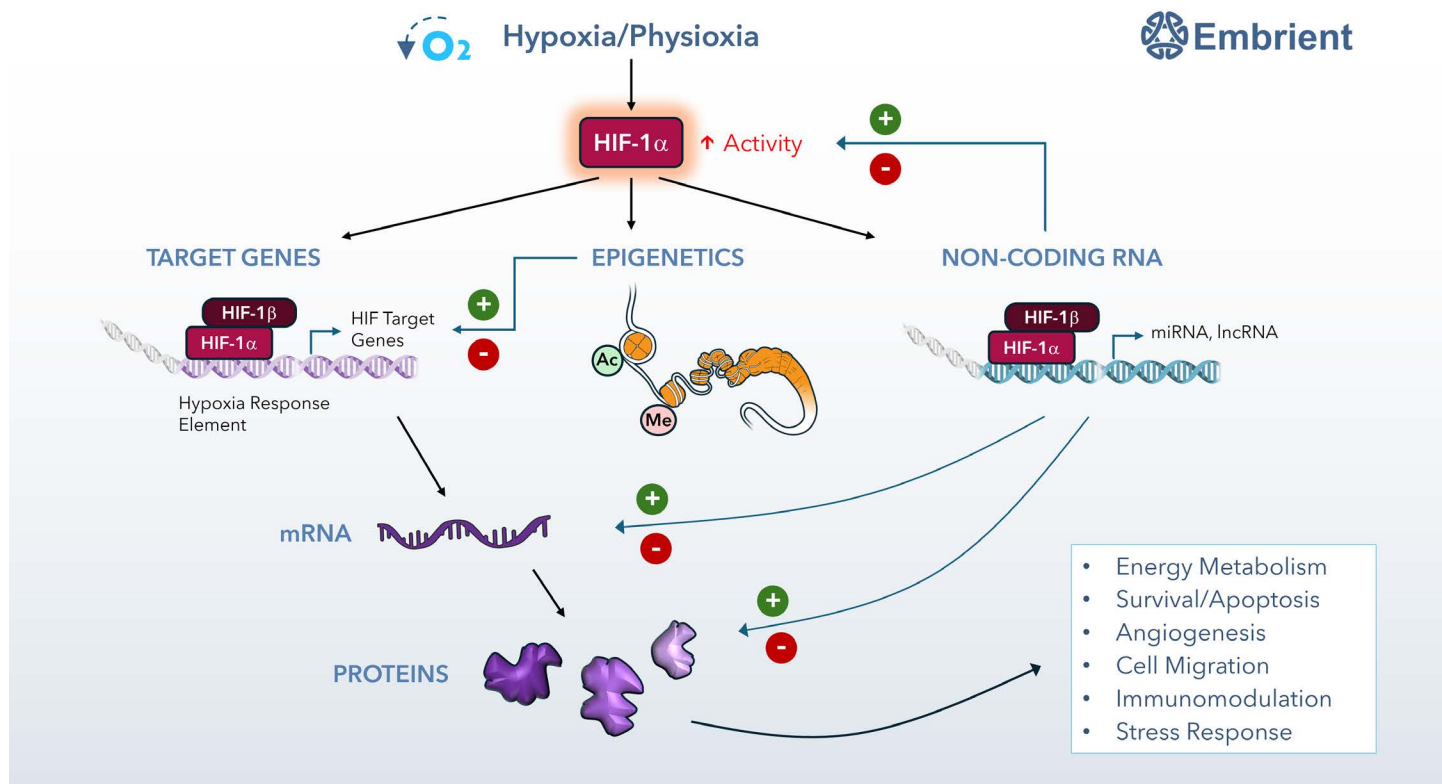


Fig. 1. A summary of the activities of HIF-1 on gene expression, epigenetics, and non-coding RNAs, and the interactions of each resulting in a complex set of diverse effects on the cellular transcriptome and proteome.

Conclusion

In conclusion, hypoxia triggers widespread transcriptomic and proteomic alterations through the activation of the HIF-1 pathway, enabling cells to thrive in low oxygen environments. These changes encompass metabolic reprogramming, angiogenesis, stress responses, epigenetics, and the modulation of non-coding RNAs, highlighting the complex interplay between gene regulation and protein expression. Understanding these dynamics provides critical insights into both physiological responses to hypoxia and pathological processes such as cancer progression, offering potential avenues for therapeutic intervention. Lastly, these findings support the concept that accurate cell culture models should be using oxygen levels that most closely resemble physiological oxygen, not the commonly used 21% atmospheric oxygen.

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