Modifying the maker: Oxygenases target ribosome biology

Qinqin Zhuang¹, Tianshu Feng², and Mathew L Coleman¹,*

¹Tumour Oxygenase Group; School of Cancer Sciences; University of Birmingham; Birmingham, UK; ²Centre for Cellular and Molecular Physiology; University of Oxford; Oxford, UK

Keywords: decoding, demethylase, hydroxylation, modification, oxygenase, ribosome, translation, 2-oxoglutarate

The complexity of the eukaryotic protein synthesis machinery is partly driven by extensive and diverse modifications to associated proteins and RNAs. These modifications can have important roles in regulating translation factor activity and ribosome biogenesis and function. Further investigation of ‘translational modifications’ is warranted considering the growing evidence implicating protein synthesis as a critical point of gene expression control that is commonly deregulated in disease. New evidence suggests that translation is a major new target for oxidative modifications, specifically hydroxylations and demethylations, which generally are catalyzed by a family of emerging oxygenase enzymes that act at the interface of nutrient availability and metabolism. This review summarizes what is currently known about the role of these enzymes in targeting rRNA synthesis, protein translation and associated cellular processes.

Introduction

Ribosome biogenesis and protein synthesis are highly orchestrated and dynamically regulated cellular processes that are tightly controlled by the modification of key regulatory factors. Modification of chromatin at rDNA loci controls rRNA production,¹ the rate limiting step of ribosome biogenesis. rRNA is itself heavily modified by base and ribose methylation and pseudouridylation, which together likely promote rRNA stability and translation efficiency.² Ribosomal proteins and translation factors are modified by phosphorylation, methylation, hypusination, dipthamide modification, and others types of modification.³⁻⁷ Such a complex array of diverse modifications have likely evolved to optimize ribosome biogenesis and translational efficiency, to promote heterogeneity in ribosome populations destined for alternative tasks,⁸ and to allow fine control of protein synthesis rate in response to nutrient availability and stress.⁹

Growing interest in a family of oxygenases that catalyze diverse oxidative modifications to DNA, RNA and protein has led to the recent discovery that the cellular machinery controlling rRNA and protein synthesis and protein translation are the target of hydroxylation and demethylation. This review aims to introduce the family of oxygenase enzymes thought to be predominantly responsible for such modifications and to summarize what is currently known about the role of oxygenases in protein synthesis.

2-Oxoglutarate-oxygenases

Oxygenases whose activities depend on Fe(II), oxygen, and the Krebs cycle intermediate 2-oxoglutarate (2OG) (‘2OG-oxygenases’) form a family of relatively poorly characterized enzymes consisting of more than 60 members in mammals.¹⁰ 2OG-oxygenases catalyze site-specific modifications, with specificity being driven by primary and secondary sequence constraints in the substrate and structural determinants within the enzyme. The catalytic domain of 2OG-oxygenases consists of a ‘double-stranded β-helix’ (DSBH), a structural arrangement that has evolved to present specific amino acid side chains within the active site to optimally co-ordinate co-factors and substrate.¹¹ A ‘facial triad’ of amino acids belonging to the conserved HXD/E motif orchestrates iron coordination (Fig. 1A). In the presence of molecular oxygen, oxidative decarboxylation of 2OG releases succinate and carbon dioxide and generates a highly reactive Fe(IV)-oxo intermediate that drives hydroxylation of the prime substrate.¹² (Fig. 1A). In eukaryotes, currently described modifications catalyzed via this mechanism are thus far limited to hydroxylation, and demethylation catalyzed via a hydroxylation reaction.¹⁰ Hydroxylation of a methyl group generally results in the formation of a highly labile hydroxymethyl intermediate that rapidly decomposes releasing formaldehyde with consequent reversal of the methyl modification (Fig. 1B). 2OG-oxygenases catalyzing demethylation include the JmjC histone demethylases, important epigenetic modifiers widely implicated in development, physiology and disease.¹³

Possibly the most well-known examples of stable hydroxylation catalyzed by 2OG-oxygenases are prolyl and lysyl modification of the extracellular matrix protein collagen,¹⁴ together with the role of hydroxylation in hypoxia signaling mediated by the Hypoxia Inducible transcription Factor (HIF).¹⁵ In the latter example, three hydroxylases (PHD1-3) modify two conserved prolyl residues in the HIFα subunit that targets it for rapid proteasomal destruction. The activities of HIFα prolyl hydroxylases are compromised under conditions in which the availability of the essential co-factor
oxygen is limited (hypoxia), leading to HIFα protein stabilization. Thus, a relatively low affinity for molecular oxygen imparts an oxygen sensing role on the HIFα hydroxylases, allowing HIFα stabilization and activation in hypoxia to drive transcriptional programs that have evolved to bring about adaption to this important physiological and pathological stress.15

The role of the HIF hydroxylases in regulating transcription has highlighted the potential for gene expression control by 2OG oxygenases. Indeed, it has since become apparent that these enzymes target the cellular machinery governing gene expression at multiple levels. For example, several 2OG oxygenases with nucleotide hydroxylase activity have now been identified, including the ALKBH family (see below) and the TET family that hydroxylate and demethylate 5-methylcytosine. The JmjC histone demethylases mediate both transcriptional activation and repression at the level of chromatins.10,13 JMJ6 catalyzes 5-lysyl hydroxylation of mRNA splicing factor U2AF65, and modulates mRNA splicing.17-20 This review will focus on recent literature describing protein synthesis as a major new target of 2OG-oxygenases.

**2OG-oxygenases Target Protein Synthesis**

Emerging evidence indicates that in addition to their role in controlling gene expression at the stages outlined above, 2OG oxygenases are also involved in translational control via modification of rDNA loci, RNAs, ribosomal proteins and translation factors.

**Histone Demethylases Regulate rRNA Transcription**

rDNA transcription is under the control of several chromatin modifiers, including members of the 2OG-oxygenase family.1 KDM2A is a mono and dimethyl histone H3 lysine 36 (H3K36me1/2) demethylase (Figs. 2 and 3) localized to the nucleolus where it binds to the rDNA promoter and represses rDNA transcription.21 KDM2B is a nucleolar H3K4Me3 demethylase (Figs. 2 and 3) that represses rDNA transcription and cell growth and suppresses tumorigenesis22 (Table 1). In contrast, PHF8 is a H3K9me1/2 demethylase (Fig. 2 and 3) that binds to the promoter region of rDNA to promote rDNA transcription.23 Thus, the earliest step in ribosome biogenesis and protein synthesis is under the control of opposing histone demethylases of the 2OG-oxygenase family. An intriguing possibility is that the JmjC demethylases could also act at later stages of
ribosome biogenesis. For example, ribosomes and chromatin both consist of charged nucleic acids in complex with small basic proteins that are often rich in lysine and arginine. Similar to histones, ribosomal proteins are subject to a range of methylations including arginine and lysine. Therefore, it is possible that nucleolar JmjC demethylases could target methylated ribosomal proteins in addition to histones. Consistent with this speculation, non-histone targets have been identified for JmjC histone demethylases.24

Nucleotide Oxygenases
AlkB is a highly conserved 2OG oxygenase in *Escherichia coli* that removes methylation adducts in DNA using a hydroxylation mechanism.25,26 It has 8 human homologues, termed ALKBH1-8. Of these, 3 have currently been implicated in targeting the protein synthesis machinery. ALKBH2 promotes rDNA transcription by repairing alkylation damage associated with rapid transcription (Fig. 3).27 ALKBH5 demethylates N6-methyladenosine (m^6^A) (Figs. 2 and 3), one of the most prevalent nucleotide modifications in mRNA and long noncoding RNA.28,29 N6-methyladenosine is recognized by specific RNA-binding proteins that modulate RNA stability, and mediates widespread gene regulation.30,31 The function of ALKBH5 m^6^A-demethylation may be related to nuclear RNA export, perhaps consistent with its nuclear localization.29 Loss of ALKBH5 is associated with defective spermatogenesis in mice, consistent with its enriched expression in the testes.29 Interestingly, m^6^A is also a target of the 2OG-oxygenase
Table 1. 2OG-oxygenases with targets in ribosome biology and protein synthesis are frequently implicated in disease, particularly cancer. It should be noted that other substrates of these enzymes may exist in other biological contexts and that the critical targets of these enzymes involved in disease are often unclear, but may include the translational targets listed. The role of JmjC family 2OG-oxygenases in disease was recently reviewed by Oppermann and colleagues.\(^\text{10}\)

| Translational Oxygenase | Translation target | Diseases |
|-------------------------|--------------------|----------|
| KDM2A                   | rDNA promoter (H3K36me1/2) | cancer |
| KDM2B                   | rDNA promoter (K3K4me3 and H3K36me1/2) | cancer |
| PHF8                    | rDNA promoter (H3K9me1/2) | cancer, mental retardation |
| ALKBH2                  | rDNA 1-meA and 3-meC | cancer |
| ALKBH5                  | mRNA N6-methyladenosine | obesity |
| FTO                     | mRNA N6-methyladenosine | obesity, cancer, Alzheimer’s, cardiovascular |
| TET1-3                  | rRNA 5-methylcytosine | cancer, neurodegeneration |
| ALKBH8                  | Arg-/Glu-tRNA (mcm5U) | — |
| TYW5                    | Phe-tRNA (yW-72) | — |
| MINA53                  | Rpl27a | cancer, asthma, autoimmunity |
| NO66                    | Rpl8 | cancer |
| OGFOD1                  | Rps23 | — |
| Jmd4                    | eRF1 | cancer |

Figure 3. Hydroxylation and demethylation events in eukaryotic ribosome biogenesis and protein translation. ALKBH2 is a demethylase that repairs alkylated rDNA. 3-meC=3-methylcytosine. 1-meA=1methyladenine. KDM2A/B and PHF8 are nucleolar histone lysine demethylases that target rDNA. MINA53 and NO66 are nucleolar histidyl hydroxylases of the large ribosomal subunit. OGFOD1 is a nuclear prolyl hydroxylase of the small ribosomal subunit. FTO and ALKBH5 are m^A RNA demethylases. TYW5 and ALKBH8 hydroxylate the anti-codon loop of the indicated tRNAs. The ? under ALKBH8 denotes an as yet unidentified mcm^U hydroxylase. Jmd4 is a hydroxylase of the translational termination factor eRF1. Note that hypoxia (red box) substantially regulates translation. Inhibition of prolyl hydroxylases in hypoxia indirectly represses EIF4E (via HIF-dependent mTOR inhibition) while activating the translation of specific transcripts via an RBM4/HIF2α/eIF4E2 cap-dependent mechanism. The orange ball represents the cap.
A common variant in the FTO gene was originally identified as a risk factor for increased BMI and predisposition to obesity (Table 1). Gene knockout studies suggest that FTO targets a specific subset of mA-containing mRNAs. FTO may target other methylated nucleotides under specific conditions, although the function of these modifications is not yet known. Since FTO is primarily expressed in the brain, and ALKBH5 in the tests, tissue-specific expression of these enzymes may avoid functional redundancy.

The TET family of 2OG-oxygenases (TET1-3) mediate epigenetic DNA modification by converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Subsequent, oxidation to 5-formylcytosine (5fC) and 5-carboxylcytosine can also bring about full reversal of the methylation. These cytosine modifications are thought to mediate their biological effects by modulating DNA duplex stability and DNA-protein (transcription factor) interactions, which in turn may in part explain the role of this subfamily of 2OG-oxygenases in cancer and neurodegeneration (Table 1). However, it is possible that other targets of TET enzymes could also be involved. Of interest here is the fact that 5mC, 5hmC and 5fC are described in databases of RNA modifications. Furthermore, recent evidence suggests that TET enzymes can indeed catalyze the formation of 5hmC in RNA (Fig. 2), raising the possibility that this class of 2OG-oxygenases could also be novel regulators of protein synthesis.

The anticodon stem and loop region of tRNA is subject to a variety of modifications that optimize tRNA folding, prevent frameshifting and ensure accurate codon selection. For example, modifications that optimize tRNA folding, prevent frameshifting and ensure accurate codon selection. Varieties of modifications that optimize tRNA folding, prevent frameshifting and ensure accurate codon selection. Furthermore, these modifications are thought to play a role in regulating tRNA stability and translation efficiency. These modifications are catalyzed by a class of enzymes known as tRNA methyltransferases.

Hydroxywybutosine, formation of which is catalyzed by the 2OG-oxygenase TYW5 (Figs. 2 and 3). However, the function of hydroxywybutosine is currently unclear. Knockdown of TYW5 did not confer a gross phenotype in HeLa cells, suggesting this modification does not play a critical role in bulk translation or cell growth, at least under normal growth conditions. It is interesting to speculate that TYW5 may be required for the efficient translation of phenylalanine-rich proteins involved in specific biological process (e.g. nucleopores), akin to the role of ALKBH8 in the DNA damage response.

**Ribosomal Oxygenases**

MINA53 is a 2OG-oxygenase that was recently assigned as a histidyl hydroxylase of the 60S large subunit protein Rpl27a (Fig. 2 and 3). MINA53 was first identified in a microarray screen for novel Myc target genes, and was subsequently shown to be required for tumor cell proliferation. MINA53 is overexpressed in some tumors relative to normal tissues, and high level of MINA53 expression may be associated with poor patient prognosis in some contexts (Table 1). However, other studies have reported that MINA53 overexpression is associated with favorable prognosis in early stages of lung cancer. Therefore, the exact role of MINA53 in tumorigenesis remains unclear, but could be highly context specific. MINA53 has also been independently studied in other contexts in addition to cancer, including allergy and immunity, where it appears to regulate T-cell differentiation (Table 1). In both cases the molecular mechanisms involved remain unclear. Although MINA53 was reported to be a demethylase of H3K9me3, detailed biochemical and structural analyses have raised questions about its biochemical activity. More recently, unbiased proteomics coupled to in vitro peptide screening identified Rpl27a as a bona fide substrate of MINA53. Rpl27a is hydroxylated in a MINA53-dependent manner on a specific histidine residue at position 39 within a HHH motif (where the hydroxylated residue is underlined) (Fig. 2). Endogenous Rpl27a purified from human and mouse cell lines, normal mouse and human tissues, and tumors is hydroxylated to near completion. The abundance of the modification may argue against a signaling role and may be more consistent with a structural function. The modified residue is located on a disordered loop that extends into the core of the ribosome. However, higher resolution ribosomal structures and functional studies are required to pinpoint the role of the HHH motif and its hydroxylation in Rpl27a function. Interestingly, Rpl27a H39 is adjacent to the residue implicated in cycloheximide binding and sensitivity in yeast, although these effects were not manifest in mammalian cells defective for MINA53 (Dr Adam Zayer, personal communication). Furthermore, MINA53 knockout mice are viable and fertile, suggesting that Rpl27a hydroxylation is not essential for normal development and reproduction.

NO66 is closely related to MINA53, sharing 39% sequence homology overall and 57% homology within the catalytic domain. Like MINA53, NO66 is also found in the nucleolus and is implicated in cancer cell growth, particularly in non-small cell lung carcinoma (Table 1). Proteomic screens coupled
to \textit{in vitro} peptide screening identified the 60S large ribosomal subunit Rpl8 as a NO66 substrate\textsuperscript{49} (Figs. 2 and 3). Consistent with the sequence conservation with MINA53, NO66 is also a histidyl hydroxylase, modifying Rpl8 at position 216 within a motif similar to Rpl27a (HQH) (Fig. 2). Endogenous Rpl8 purified from human and mouse cell lines, normal mouse and human tissues, and tumors is hydroxylated to near completion.\textsuperscript{49} Similar to Rpl27a, the hydroxylated residue is within a disordered loop that extends into the ribosome, but in this case it is proximal to the peptidyl-transferase center (PTC), perhaps suggestive of an important role in translation. Mutation of the corresponding residue in yeast Rpl8 (Rpl2) affects peptidyl-tRNA binding, PTC activity and confers resistance to the antibiotic sparsomycin.\textsuperscript{65} However, chronic NO66 knockdown in human cells does not appear to drastically affect polysome profiles, cell growth or sparsomycin sensitivity in our hands (Tianshu Feng, personal communication). This disparity could reflect the difference between non-conservative mutation and a relatively subtle modification. However, it does suggest that PTC activity is unlikely to be grossly affected by Rpl8 hydroxylation. Perhaps NO66 activity and Rpl8 hydroxylation regulate the translation of specific mRNA’s or simply fine tune the structural integrity of the ribosome in the vicinity of the PTC.

In prokaryotes, a 2OG oxygenase named YcFD that is highly related to NO66 catalyzes arginyl hydroxylation of the 60S ribosomal protein L16 at position 81\textsuperscript{49} (Fig. 2). Structural and phylogenetic analyses indicate that NO66 was likely evolved from YcFD, and MINA53 from NO66 in a much later gene duplication event.\textsuperscript{49,60} Similar to MINA53 and NO66, the function of Rpl16 hydroxylation is unknown. Rpl16 R81 hydroxylation is essentially complete in wildtype strains and absent in YcFD gene knockouts.\textsuperscript{49} Surprisingly, both knockout and overexpression of YcFD are associated with reduced growth potential under some circumstances.\textsuperscript{49,66}

In addition to NO66 and MINA53, a third eukaryotic ribosomal protein hydroxylase was recently identified. The 2OG-oxygenase OGFOD1 is distantly related to the HIF prolyl hydroxylases, and catalyzes hydroxylation of an evolutionary conserved prolyl residue in Rps23, a component of the 40S small subunit (Figs. 2 and 3). Interestingly, Rps23 is doubly hydroxylated by the OGFOD1 ortholog in yeast and algae\textsuperscript{69}, but only singly hydroxylated in higher eukaryotes.\textsuperscript{67,68} Similar to Rpl27a and Rpl8, Rps23 hydroxylation is essentially complete in all cells and tissues. The target prolyl residue, corresponding to Pro62 in humans, is located at the apex of a loop that projects into the decoding center of the ribosome, which led to the postulation that hydroxylation is required for optimal translational accuracy.\textsuperscript{68,69} Using reporters of stop codon decoding as a measure of translational accuracy it was shown that the inhibition of OGFOD1 orthologs has variable effects on stop codon readthrough\textsuperscript{68,69}. OGFOD1 inhibition modestly enhances translational termination in human and \textit{Drosophila} cells\textsuperscript{67,68}, whereas more dramatic effects were observed in yeast in a bidirectional manner that was highly context-specific.\textsuperscript{69} Despite observing no measurable loss in translational termination efficiency, inhibition of OGFOD1 in \textit{Drosophila} and human cells is often associated with marked translational arrest phenotypes including: reduced protein synthesis; increased elf2\textalpha phosphorylation; stress granule formation and autophagy.\textsuperscript{67,68} Therefore, the role of altered translational termination in the phenotypes reported was questioned. It would be of interest to investigate whether other measures of translational accuracy and decoding might explain the growth deficits observed. Conversely, the deletion of OGFOD1 ortholog Tpa1 in yeast resulted in substantial changes in translational termination.\textsuperscript{69} Although associated growth alterations were not reported, Tpa1 knockout cells are viable, which suggests that levels of endogenous stop codon readthrough were compatible with growth in this context.

Further investigation is required to determine whether deregulated ribosomal hydroxylase activity drives diseases associated with these enzymes (such as cancer), or explains the complex phenotypes associated with enzyme ablation in eukaryotes. Some 2OG-oxygenases have multiple substrates, raising the possibility that other targets may also exist that contribute to the role of these enzymes in physiology and disease.

### Translation Factor Hydroxylases

The first example of a hydroxylated translation factor was discovered in the context of hypusine, a uniquely modified amino acid only found in elf5\textalpha\textsuperscript{50} (Fig. 3). Hypusine is formed by the transfer of an n-butylamine group from spermidine to the lysyl side chain, followed by hydroxylation.\textsuperscript{70,71} In this case hydroxylation is catalyzed by a unique enzyme that is structurally distinct to 2OG-oxygenases. Deoxyhypusine hydroxylase is a HEAT-repeat-containing dinuclear iron enzyme that catalyzes the final step in hypusine formation in an oxygen- and Fe(II)-dependent,\textsuperscript{72,73} but 2OG-independent,\textsuperscript{74} manner.

Consistent with a fundamental role in protein synthesis, elf5\textalpha has been shown to promote elongation and the translation of polyproline motifs.\textsuperscript{75,76} Importantly, the hypusine modification of elf5\textalpha is essential for its function,\textsuperscript{75-78} perhaps related to the proximity of the modification to the acceptor stem of the P-site tRNA.\textsuperscript{75} The importance of elf5\textalpha in protein synthesis and eukaryotic development and viability is underscored by its evolutionary conservation, with a homolog also present in bacteria (EF-P). Interestingly, the lysine residue that is modified to hypusine in elf5\textalpha is conserved in EF-P, where it is also subject to an unusual modification.\textsuperscript{79} Lysine 34 is post-translationally modified by a \(\beta\)-lysine residue. Importantly, EF-P and elf5\textalpha modifications share additional similarities. Following lysinylation, lysine 34 is modified by a hydroxylase termed YfcM,\textsuperscript{80} which is distinct from the elf5\textalpha HEAT-repeat metalloenzyme and structurally unrelated to 2OG-oxygenases.\textsuperscript{81} Similar to elf5\textalpha, EF-P has been implicated in elongation and translation of polyproline tracts.\textsuperscript{82,83}

Recent evidence suggests that the regulation of elongation factors by hydroxylation extends beyond EF-P/elf5\textalpha. Structure-directed bioinformatics analyses identified a 2OG-oxygenase in \textit{Pseudomonas} related to the HIF prolyl hydroxylases, which was subsequently shown to target the EF-Tu elongation factor\textsuperscript{84} (Fig. 2). EF-Tu delivers aminoacyl-tRNA to the ribosome and
Decoding: A Key Target of 2OG-oxygenases in Translation?

Although the hydroxylation and demethylation events reviewed here have been discovered across ribosome biology, common roles may be emerging in elongation and decoding. With respect to decoding, 3 hydroxylation events described above are directly linked to translational fidelity; (i) Rps23 proline 62 hydroxylation (OGFOD1), (ii) anti-codon loop hydroxylation of tRNAs (TYW5, ALKBH8), and (iii) N-domain hydroxylation of eRF1 (Jmjd4). It is of interest to highlight the latter 2 examples, where independent hydroxylases target the codon reading domains of tRNA and a peptidyl tRNA mimic. Thus, at least within the limits of the studies published to date, 2 hydroxylations may be present in the decoding center of the ribosome at any one time, with hydroxylated Rps23 proline 62 in the proximity of eRF1 K63 hydroxylation or tRNA hydroxylated at wybuto sine (TYW5) or mcm5U (ALKBH8). Interestingly, an obligate partner of the methyltransferase activity of ALKBH8 (Tm112)42 is also required for the activity of a methyltransferase that targets eRF1,98 raising the possibility that there may be cross-talk between eRF1 and tRNA modifications. Considering that an unidentified tRNA mcm5U exists44 (see above), and many members of the 2OG-oxygenase family remain poorly or completely uncharacterised,10 it is possible that other examples of hydroxylation targeting decoding may emerge.

Decoding is considered to be a major determinant of biological ‘fitness’ and as such is a highly evolved process.99,100 Decoding requires fast and accurate selection of the correct tRNA from a pool of competitors and involves conformational changes to both the ribosome and the tRNA (or its mimic). Perhaps oxygenases have allowed evolution to fine tune the architecture of the ribosome using hydroxylation, a relatively subtle modification, which could optimize protein-protein and protein-RNA interactions, efficient codon recognition and/or conformational rearrangements. Alternatively, the oxidative modifications described here may allow decoding to sense nutrient availability via oxygenase activity. Interestingly, reduced translational fidelity can be
advantageous under some circumstances. For example, decoding errors can promote adaption in response to stress in bacteria.101 Perhaps a collective reduction in decoding center hydroxylation under conditions of stress (e.g., amino acid starvation, metabolic flux, and/or hypoxia) could reduce translational fidelity to signal adaptive responses. In such a scenario, the oxygenases would be acting as sensors relaying changes in nutrient availability and metabolism to ribosomal decoding.

Translational Oxygenases as Sensors

The cofactor requirements of 2OG-oxygenases (2OG, Fe(II), O2) place them at a unique interface between nutrient availability and metabolism (Fig. 1). Enzymes with a relatively low affinity for one or more co-factors have the potential to act as sensors of that nutrient (and its metabolic predecessors). The clearest example of a sensing role thus far is for the hypoxia-responsive HIF system, as outlined above. Indeed, hypoxia is well-known to have multiple profound effects on translation control9 (Fig. 1). There is a substantial decrease in eLF4E-driven cap-dependent translation in hypoxia, at least partly due to mTOR inhibition via HIF-dependent and -independent pathways.102,103 Translation of proteins involved in the adaptive response to hypoxia is maintained via IRES control9 and a novel eLF4E2-mediated cap-dependent mechanism that involves direct binding of HIF2α104. Therefore, HIF hydroxylases may contribute to translational control indirectly via HIF regulation, and perhaps more directly via alternative substrates (such as those regulating eEF2 phosphorylation).

HIF-independent but oxygen-sensitive mechanisms of translational control could be of interest with respect to the wider family of 2OG-oxygenases. Examples of translational oxygenases acting as oxygen sensors are currently lacking however. Although MINA53, NO66, OGFOD1 and Jmjd4 all require oxygen for hydroxylation of their respective substrates they can maintain efficient catalysis under conditions of severe hypoxia.49,68,69,89 Whether other enzymes discussed here (e.g. RNA hydroxylases/ demethylases) could be more sensitive to oxygen starvation is not yet known. For those enzymes that have been tested and found to be relatively insensitive to hypoxia it is possible that a concurrent reduction in one or more other co-factors could cause a more dramatic loss in activity. It is interesting to note that the EF-Tu prolyl hydroxylase of Pseudomonas may have a relatively high Km for Fe(II),84 raising the possibility that this and perhaps other translational hydroxylases may sense Fe(II), and thereby link Fe(II) availability to translational control. Furthermore, some 2OG-oxygenases are competitively inhibited by intermediates of the TCA cycle such as fumarate and succinate (‘oncometabolites’, Fig. 1B), which are elevated in diseases associated with fumarate hydratase and succinate dehydrogenase deficiency, respectively.105,106 Neomorphic mutations in isocitrate dehydrogenases lead to 2-hydroxyglutarate production (Fig. 1B) in glioblastomas and acute myeloid leukemia cancers, which is associated with variable effects on the activity of some 2OG-oxygenases.105-108 It is possible that translational oxygenases might also communicate these metabolic disturbances to gene expression control at the level of protein synthesis. Such disturbances in co-factor availability and metabolism could cause a significant reduction in a specific hydroxylation or demethylation event within translation, or perhaps more modest effects on multiple modifications that collectively modulate protein synthesis.

Since many of the oxidative modifications described here go to near completion (e.g., ribosomal proteins and eRF1), how quickly would a reduction in 2OG-oxygenase activity due to reduced co-factor availability lead to a loss of the modification and a biological response? Reversal of some post-translational modifications can lead to rapid loss of the modification in the absence of the forward reaction. However, although theoretically feasible, a reversal enzyme for hydroxylation has yet to be described. Isotopic labeling and mass spectrometry experiments indicated that hydroxylation catalyzed by a HIF asparaginyl hydroxylase is unlikely to be reversed.109 Therefore, a reduction in hydroxylation following co-factor depletion would rely on natural turnover of the substrate, with relatively stable substrates only eliciting altered biological responses following chronic nutrient depletion (such as those found in pathological conditions for example).

Future Perspectives

Taken together, the literature reviewed here supports protein synthesis as a major new target of 2OG-oxygenases. Since many of these enzymes remain uncharacterized, and those that have been studied have the potential to target multiple substrates, it seems likely that the list of translational oxygenases will continue to grow. These may also be complemented by novel classes of hydroxylases as exemplified by the eIF5α HEAT-repeat hydroxylase and the YfcM hydroxylase of EF-P. Although current examples of hydroxylases targeting the translational machinery appear to be enriched within elongation and decoding, it is possible that oxygenases will also be discovered that target other key regulatory steps such as initiation and recycling.

2OG-oxygenases are commonly deregulated in disease10 (see also Table 1), likely due to their action at the interface of nutrient availability and metabolism, and their common role in gene expression and growth control. These enzymes have small druggable active sites that are amenable to small molecule inhibition.110 As such, 2OG-oxygenases are attracting significant interest as novel therapeutic targets. Considering the role of protein synthesis in disease, further work characterizing the role of 2OG-oxygenases in translation and their potential as drug targets is warranted. This should include attempts to clarify the function of those modifications reviewed here where the physiological significance remains unclear, together with efforts aimed at discovering novel translational oxygenases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors would like to thank Dr Matthew Cockman for critical reading of the manuscript.
The research in the Coleman laboratory is funded by an MRC New Investigator Award. Tianshu Feng is supported by a Claren-

don Award (University of Oxford).

References

1. Gulner RM, Lomberk G. Epigenetic control of RNA poly-


dermase I transcription in mammalian cells. Biochim Bi-



ophys Acta 2002; 1579:39-404; PMID:12062748; http://dx.doi.org/10.1016/S0005-2760(02)00104-4

2. Watkins NJ, Bohnack MT. The box C/D and H/ACA snoRNP: key players in the modification, processing and the dynamic folding of ribosomal RNA. Wiley Interdiscip Rev RNA 2012; 3:397-414; PMID:2262652; http://dx.doi.org/10.1002/wrna.120.117

3. Clarke SG. Protein methylation at the surface and


in gene regulation and organismal biology. Nature


2005; 436:207-14; PMID:15901537; http://dx.doi.


org/10.1038/nature03933

4. Graille M, Figaro S, Kervestin S, Buckingham RH, 



and the dynamic folding of ribosomal RNA. Wiley


2012; 94:1553-43; PMID:23060654; http://dx.doi.org/10.1002/8098510

5. Kamita M, Kimura Y, Ino Y, Kamita M, Kimura Y, Ino Y, Kamp RM, Polevoda B, 



6. Pierrat OA, Mikitova V, Bush MS, Browning KS, 



7. Klose RJ, Kallin EM, Zhang Y. JmjC-domain-con-


domaining A, Dimmel S. Junon protein domain-containing gene protein 6 (Jmd6) is required for angiogenic sprouting and regu-

lates splicing of VEGF-receptor 1. Proc Natl Acad Sci U S A 2011; 108: 3276-81; PMID:21308899; http://dx.


org/10.1073/pnas.1013576

8. Bobek JN, Guarian V, Koyanagi M, Roewe T, Leng-


deling A, Schermuly RT, Gellert P, Braun T, Zecher A, 



9. Spriggs KA, Bushell M, Willis AE. Translational regu-

lation of gene expression during conditions of cell


mortality. Mol Cell 2008; 30:393-402; PMID:18498744; 

10. Johansson C, Tumber A, Che K, Cain P, Nowak R, 



11. McDonough MA, Loenarz C, Chowdhury R, Clifton 



12. Hewison KS, Granatino N, Welford RW, McDona-



13. Johansson C, Tumber A, Che K, Cain P, Nowak R, 



14. Myllyharju J, Kivirikko KI. Collagens, modifying enzymes


15. Kaelin WG Jr., Ratcliffe PJ. Oxygen sensing by meta-



16. Delatte B, Deplus R, Fuki F. Playing TETris with


dNA modifications. EMBO J 2014; 33:1198-211; PMID:24825349; http://dx.doi.org/10.1002/num.22053

17. Barman-Aksozen J, Beguin C, Dogan AM, Schneider-



18. Boeckel JN, Guarian V, Koyanagi M, Roewe T, Leng-


deling A, Schermuly RT, Gellert P, Braun T, Zecher A, 



19. Hein A, Grimm C, Muller U, Hausasser S, Mackeen 



20. D origins of tRNA genes. Nucleic Acids Res 1998; 26: 148-



21. Tanaka Y, Okamoto K, Teye K, Uemata T, Yamagawa 



22. Frescas D, Guardavaccaro D, Bassermann F, Koyama-



23. Feng W, Yonezawa M, Ye J, Jennewein T, Grumm I, 



24. Gerken T, Giraud CA, Dogar AM, Schneider A, 



25. Duncan T, Trewick SC, Koivisto P, Bates PA, Lin-



26. Fukuyama T, Hashimoto Y. Regulation of the fat mass and obesity associated gene (FTO) by methylation. Adv Genet 2013; 82: 171-97; PMID:23578552; http://dx.doi.org/10.1007/102573962; http://dx.doi.org/10.1038/rec.2009.209

27. Li P, Gao S, Wang L, Yu F, Li J, Wang C, Li J, Wong J. ABCH2 couples regulation of ribosomal DNA trans-

cription with DNA alkylation repair. Cell reports 2013; 4:817-29; PMID:23729924; http://dx.doi.org/10.


10.1016/j.10973177

28. Funes PO, Johanns RF, Seegert E. AlkB-mediated oxy-


dative demethylation reverses DNA damage in Escher-


29. Cunningham RL, Glaister IA, Schorichter-Deshamia P, 



30. Cuttler JE, Tang Y. A heritable and common variant in 



31. Li P, Gao S, Wang L, Yu F, Li J, Wong J. ABCH2 couples 



32. Gulati P, Yeo GS. The roles of Jumonji-type hy-



33. Clarke SG. Protein methylation at the surface and



34. Gerken T, Giraud CA, Dogar AM, Schneider A, 



35. Wu H, Zhang Y. Reversing DNA methylation: mecha-



36. Li CJ, Vagbo CB, Shi Y, Wang WL, Song SH, et al. 



37. Zheng G, Dahl JA, Niu Y, Fedorcek P, Huang CM, 



38. Songe-Moller L, van den Born E, Leihne V, Vagbo 



39. El Yacoubi, B, Bailly, M, de Crecy-Lagard, V. Biosyn-



40. Duncan T, Trewick SC, Koivisto P, Bates PA, Lin-



41. Li P, Gao S, Wang L, Yu F, Li J, Wang C, Li J, Wong J. ABCH2 couples regulation of ribosomal DNA trans-

cription with DNA alkylation repair. Cell reports 2013; 4:817-29; PMID:23729924; http://dx.doi.org/10.


10.1016/j.10973177

42. Songe-Moller L, van den Born E, Leihne V, Vagbo 



Funding

The research in the Coleman laboratory is funded by an MRC New Investigator Award. Tianshu Feng is supported by a Claren-

don Award (University of Oxford).
biogenesis of multiple wobble uridine modifications implicated in translated decoding. Mol Cell Biol 2010; 30:1814-27; PMID:20123966; http://dx.doi.org/10.1128/MCB.01602-09

43. Fu XM, Li, Zhang W, Ren J, Pan T, He C. The AlkB domain of mammalian ARH8 catalyzes hydroxylation of 5-methoxycarbonylaminoleucine at the wobble position of tRNA. Angew Chem Int Ed Engl 2010; 49:8885-8; PMID:20583019; http://dx.doi.org/10.1002/anie.201005198

44. van den Born E, Vagbo CR, Sorge-Moller L, Lehne V, Lien F, Leszczyńska G, Malkiewicz A, Krokán HE, Kirkepik F, Klangland A, et al. ALKBH1-mediated formation of a novel diastereomeric pair of wobble nucleotides in mammalian tRNA. Nat Commun 2011; 2:1772; PMID:21285950; http://dx.doi.org/10.1038/ncomms1173

45. Belegy-U davaya M, Patil A, Rooney JP, DiRenzo D, Young CM, Costikin DL, Zitomer RS, Belegy-T. 133 54. Teye K, Tsuneoka M, Arima N, Koda Y, Nakamura K, Sueoka-Aragane N, Sato A, Hisatomi T, Noma A, Ishitani R, Kato M, Nagao A, Nureki O, Noma A, Kirino Y, Ikeuchi Y, Suzuki T. Biosynthesis of squamous cell carcinoma. Clin Cancer Res 2013; 19:1585-97; PMID:23874603; http://dx.doi.org/10.1158/1078-0432.CCR-13-0543

46. Mori T, Okamoto K, Tanaka Y, Teye K, Umata T, Ohueda K, Tsuchiya K, Okabe M, Tsuneoka M. Ablation of MinC reduces cell death in response to the airways. Sci Transl Med 2013; 15:1855-7; PMID:23748603; http://dx.doi.org/10.1126/scitranslmed.3300566

47. Okamoto M, Van Stry M, Chung L, Koyanagi M, Sun X, Suzuki T, Ohara O, Kimura H, Hikjuka A, Kabe H. Targeting translation elongation factor 2 suppressors, controls T helper type 2 bias. Nat Immunol 2010; 11:979-86; PMID:19651615; http://dx.doi.org/10.1038/ni.1747

48. Yosif N, Shaile AK, Gaaljumite BN, Hl H, Lee Y, Awashti A, Wu C, Kawaz K, Xiao S, Jorgolli M, et al. Dynamic regulatory network controlling TH cell differentiation. Nature 2013; 496:461-8; PMID:23467088; http://dx.doi.org/10.1038/nature12981

49. Lu Y, Chang Q, Zhang Y, Beekhold Z, Rajanakusay Z, Zhan H, Castetonn V, Shi X, Chen F. Lung cancer-associated JmC. Mol Biol Cell 2012; 23:6262-73; PMID:22703059; http://dx.doi.org/10.1091/mbc.E12-07-0542

50. Elrichj B, Reichenjzer M, Herg M, Scholl M, Heid H, Storj WW, Schmidt-Zachmann MS. NO66, a highly conserved dual location protein containing multiple WWP1 and WWP2 domains, is a novel therapeutic target oncogene for lung cancer. Mol Cancer Ther 2007; 6:3211-21; PMID:17591763; http://dx.doi.org/10.1158/1535-7163.MCT-06-0659

51. Abbruzzese A, Park MH, Folk J. Deoxyhypusine hydroxylase from rat testis. Partial purification and characterization. J Biol Chem 1997; 272:3085-9; PMID:9947671

52. Gutierrez E, Shin BS, Woolserenhule CJ, Kim JR, Saini P, Buskirk AR, Dever TE. EF-P promotes translation of polyribosomal mRNAs. Mol Cell 2013; 51:35-45; PMID:23572016; http://dx.doi.org/10.1016/j.molcel.2013.04.021

53. Saini P, Tyler DE, Green R, Dever TE. Hypusin-containing protein eIF5A promotes translation elongation. Nature 2009; 462:483-9; PMID:19706422; http://dx.doi.org/10.1038/nature08384

54. Park MH. The essential role of hypusine in eukaryotic translation initiation factor 4E (eIF-4E). Purification of eIF-4E and its precursors and comparison of their activities. J Biol Chem 1989; 264:18531-5; PMID:5295461

55. Park MH, Wolf C, Smir-McBride Z, Hershey JW, Folk JE. Comparison of the activities of variant forms of eIF-4E. The requirement for hypusine or deoxyhypusine. J Biol Chem 1991; 266:7988-94; PMID:1850732

56. Aoki H, Xu J, Emili A, Chosay JG, Golshan A, Ganoza MC. Interactions of elongation factor EF-P with the Escherichia coli ribosome. Mol Microbiol 2008; 69:275-91; PMID:18201202; http://dx.doi.org/10.1111/j.1462-2920.2007.05628.x

57. Pei L, Starostal A, Virumke A, Kitchinson GC, Ten- son T, Remme J, Wilson DN. Lynch syndrome translation elongation factor EF-P is hydroxylated by Vma7. Nat Chem Biol 2012; 8:695-7; PMID:22076199; http://dx.doi.org/10.1038/nchembio.1001

58. Kobayashi K, Katz A, Rajovic A, Ishii R, Bramson OE, Fritsas MA, Ishiniy Rilla M, Nurkute O. The non-canonical hydroxylase structure of Vma7 reveals a metal ion-coordination motif required for EF-P hydroxylation. Nucleic Acids Res 2014; 42:12295-305; PMID:25277439

59. Pei L, Starostal A, Rusak J, Kitchinson GC, Virumke A, Spierer M, Tenson T, Jungk K, Remme J, Wilson DN. Distinct XPPX sequence motifs induce ribosome stalling, which is rescued by the translation elongation factor EF-P. Proc Natl Acad Sci U S A 2013; 110:15265-70; PMID:24083312; http://dx.doi.org/10.1073/pnas.1310642110

60. Ude S, Rusak J, Starostal A, Krabinger T, Wilson DN, Jungk K. Translation elongation factor EF-P alleviates ribosome stalling at polypeptide stretches. Sci 2013; 339:82-5; PMID:23239583; http://dx.doi.org/10.1126/science.1228985
84. Scotti JS, Leung IK, Ge W, Bentley MA, Paps J, Kramer HB, Lee J, Aik W, Choi H, Paulsen SM, et al. Human oxygen sensing may have origins in prokaryotic elongation factor Tu prolyl-hydroxylation. Proc Natl Acad Sci U S A 2014; 111:13351-6; PMID:25197067; http://dx.doi.org/10.1073/pnas.1409916111

85. Kavalaukas D, Nissen P, Knudsen CR. The busiest of all ribosomal assistants: elongation factor Tu. Biochemistry 2012; 51:2642-51; PMID:22492071; http://dx.doi.org/10.1021/bi300077s

86. Kaul G, Pattan G, Rafeequi T. Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. Cell Biochem Funct 2011; 29:227-34; PMID:21394738; http://dx.doi.org/10.1002/cbf.1740

87. Kenney JW, Moore CE, Wang X, Proud CG. Eukaryotic elongation factor 2 kinase, an unusual enzyme with multiple roles. Adv Biol Regul 2014; 55:15-27; PMID:24853990; http://dx.doi.org/10.1016/j.abr.2014.04.001

88. Romero-Ruiz A, Baustia L, Navarro V, Heras-Garvin A, March-Diaz R, Castellano A, Gomez-Diaz R, Castro MJ, Berza E, Lopez-Barnojo J, et al. Prolyl hydroxylase-dependent modulation of eukaryotic elongation factor 2 activity and protein translation under acute hypoxia. J Biol Chem 2012; 287:9651-8; PMID:22543865; http://dx.doi.org/10.1016/j.jbc.2012.04.003

89. Nakamura Y, Ito K. tRNA mimicry in translation termination. RNA 2012; 18:1210-21; PMID:22543865; http://dx.doi.org/10.1016/j.rna.031997.111

90. Nakamura Y, Ito K. tRNA mimicry in translation termination. RNA 2012; 18:1210-21; PMID:22543865; http://dx.doi.org/10.1016/j.rna.031997.111

91. Pan-Minogue H, Du M, Pisarev AV, Kallmeyer AK, Salas-Marcio J, Keeling KM, Thompson SP, Pestova TY, Brockwell DM. Distinct eRF3 Requirements Suggest Alternate eRF1 Conformations Mediate Peptide Release during Eukaryotic Translation Termination. Molecular Cell 2008; 30:359-69; PMID:18538658; http://dx.doi.org/10.1016/j.molcel.2008.03.020

92. Pan-Minogue H, Du M, Pisarev AV, Kallmeyer AK, Salas-Marcio J, Keeling KM, Thompson SP, Pestova TY, Brockwell DM. Distinct eRF3 Requirements Suggest Alternate eRF1 Conformations Mediate Peptide Release during Eukaryotic Translation Termination. Molecular Cell 2008; 30:359-69; PMID:18538658; http://dx.doi.org/10.1016/j.molcel.2008.03.020

93. Feng T, Yamamoto A, Wilkins SE, Sokolova E, Yates 3rd J. HemK2 protein, encoded on human chromosome 21, methylates translation termination complexes containing eRF1–eRF3 or eRF1–ABCE1. Cell Rep 2014; 8:59-65; PMID:25001285; http://dx.doi.org/10.1016/j.celrep.2014.04.016

94. Frolova L, Seitz-Nebi A, Kisselev L. Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. RNA 2002; 8:129-36; PMID:11911360; http://dx.doi.org/10.1017/S1355838200000777

95. Frolova L, Seitz-Nebi A, Kisselev L. Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. RNA 2002; 8:129-36; PMID:11911360; http://dx.doi.org/10.1017/S1355838200000777

96. Kaul G, Pattan G, Rafeequi T. Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. Cell Biochem Funct 2011; 29:227-34; PMID:21394738; http://dx.doi.org/10.1002/cbf.1740

97. Preis A, Heuer A, Barrio-Garcia C, Hauser A, Eyler DE, Berninghausen O, Green R, Becker T, Beckmann R. Cryoelectron microscopic structures of eukaryotic translation termination factor eRF1. FEBS Lett 2008; 582:2352-6; PMID:18539146; http://dx.doi.org/10.1016/j.febslet.2008.04.088

98. Schwinger S, Pearson TR, Wang X, Proud CG. Functional and structural insights into the RNA binding domain of human translation termination factor eRF1. RNA 2003; 9:1611-21; PMID:12777372; http://dx.doi.org/10.1017/S1355838200000777

99. Ogle JM, Ramakrishnan V. Structural insights into the invariant uridine of stop codons contacts the conserved NIKSR loop of human eRF1 in the ribosome. EMBO J 2002; 21:5302-11; PMID:12356746; http://dx.doi.org/10.1093/emboj/cdf484

100. Wohlgemuth I, Pohl C, Mittelstaet J, Konevega AL, Rodgers MA, et al. Oncometabolites-driven tumorigenesis: From genetics to targeted therapy. Int J Cancer 2014; 135:2237-48; PMID:25124653; http://dx.doi.org/10.1002/ijc.29080

101. Reynolds NM, Lazazera BA, Iba M. Cellular mechanisms that control mistranslation. Nat Rev Microbiol 2010; 8:809-56; PMID:21079653; http://dx.doi.org/10.1038/nrmicro2472

102. Arsham AM, Howell JJ, Simon, MC. A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. J Biol Chem 2003; 278:29655-60; PMID:12777372; http://dx.doi.org/10.1017/S1355838200000777

103. Jacob MD, Fabian MR, Payette J, Holcik M, Paus A, Lee S. An oxygen-regulated switch in the protein synthesis machinery. Nature 2012; 486:126-9; PMID:22678294

104. Morin A, Letouze E, Gimenez-Roqueplo AP, Favier J. Oncometabolites-driven tumorigenesis: From genetics to targeted therapy. Int J Cancer 2014; 135:2237-48; PMID:25124653; http://dx.doi.org/10.1002/ijc.29080

105. Morin A, Letouze E, Gimenez-Roqueplo AP, Favier J. Oncometabolites-driven tumorigenesis: From genetics to targeted therapy. Int J Cancer 2014; 135:2237-48; PMID:25124653; http://dx.doi.org/10.1002/ijc.29080

106. Yang M, Soga T, Pollard PJ. Oncometabolites: linking altered metabolism with cancer. J Clin Invest 2013; 123:6352-8; PMID:23999438; http://dx.doi.org/10.1172/JCI67228

107. Kaolin WG Jr, Mc Knight SL. Influence of metabolism on epigenetics and disease. Cell 2013; 153:56-69; PMID:23540690; http://dx.doi.org/10.1016/j.cell.2013.03.004

108. Loman JA, Kaolin WG Jr. What a difference a hydroxyl makes: mutant IDH1, (R)-2-hydroxyglutarate, and cancer. Genes Dev 2013; 27:836-52; PMID:23630074; http://dx.doi.org/10.1101/gad.217406.113

109. Singleton RS, Trudgian DC, Fischer R, Kessler BM, Rambow PJ, Cockman ME. Quantitative mass spectrometry reveals dynamics of factor-inhibiting hypoxia-inducible factor-catalyzed hydroxylation. J Biol Chem 2003; 278:29655-60; PMID:12777372; http://dx.doi.org/10.1017/S1355838200000777

110. Rose NR, McDonough MA, King ON, Kawamura A, Schiefel CJ. Inhibition of 2-oxoglutarate dependent oxygenases. Chem Soc Rev 2011; 40:4364-97; PMID:21930591; http://dx.doi.org/10.1039/c00203h