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Opinion

A formyl axis links metabolism to translation

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Initiator methionine tRNA is a key regulator of translation and cell growth. Recent work places formyl chemistry as a regulatory nexus across three molecular layers. In the protein layer, N-formylmethionine supports initiator tRNA recognition and stress-responsive proteostasis pathways. In the RNA layer, 5-formylcytidine at C34 of mitochondrial $tRNA^{Met}$ expands AUG/AUA decoding. In the DNA layer, 5-formylcytosine activates polymerase III transcription of $tRNA_i^{Met}$ gene clusters during frog zygotic genome activation. Despite distinct formylation routes and readouts, these layers converge on translational regulation and share metabolic dependencies. One-carbon metabolism supplies methyl/formyl precursors, while mitochondrial and oxidative states influence formyl-mark installation through redox balance and α-ketoglutarate-dependent dioxygenase activity. Together, these layers suggest a formyl-dependent rheostat linking metabolic state to translation capacity.

Highlights

The formyl group (–CHO) functions as a regulatory modification of translation initiation and the biology of initiator methionine at three molecular levels—protein, RNA, and DNA.

N-formylmethionine defines start-site identity; 5-formylcytidine in RNA expands mitochondrial $tRNA^{Met}$ decoding; 5-formylcytosine in DNA epigenetically activates *initiator tRNA* gene transcription.

All three formyl modifications derive their formyl carbon from one-carbon metabolism and their efficient installation depends on mitochondrial metabolic state.

The formyl axis is proposed as a rheostat coupling metabolic state to translation capacity.

Translation initiation at metabolic crossroads

Of the many steps in gene expression, translation initiation is the point at which the cell irreversibly commits ribosomes, energy, and amino acids to the production of a specific protein. This decision, once taken, consumes a substantial fraction of the cell's metabolic budget. In rapidly growing cells, translation consumes more ATP than any other biosynthetic process [1], and the rate of translation initiation is a primary determinant of both global protein output and cell proliferation capacity [2]. Hence, translation initiation is tightly controlled by nutrient-sensing signaling networks that couple cellular growth decisions to metabolic state [3]. This coupling ensures that cells only commit to new protein synthesis when metabolic resources are available to sustain it.

Translation initiation is intimately linked to methionine, which serves as the universal initiator of protein synthesis across all domains of life and is also deeply intertwined with **one-carbon metabolism (OCM**; see Glossary) [4]. Methionine is converted to **S-adenosylmethionine (SAM)**, the universal methyl donor for DNA, RNA, histones, and RNA modifications, and is a key regulator of mTOR (mechanistic Target of Rapamycin) signaling, driving translation and ultimately cell proliferation [5]. Methionine therefore, connects one-carbon flux, methyl-donor availability, and growth control. Significantly, methionine restriction inhibits translation initiation while simultaneously promoting healthy aging by modulating tyrosine degradation pathways [6].

At the molecular level, translation initiation capacity depends on the availability of **initiator methionine tRNA** [**$tRNA_i^{Met}$** in the cytosol and **mitochondrial methionine tRNA (mt-$tRNA^{Met}$)** in mitochondria]. This specialized tRNA recognizes start codons, and its abundance can influence the frequency with which new polypeptide chains are initiated [7]. In both normal physiology and cancer, $tRNA_i^{Met}$ levels are dynamically regulated and correlate with proliferative capacity [8–10]. The biology of $tRNA_i^{Met}$ thus sits at the intersection of metabolism and gene expression.

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It recently emerged that a single chemical motif, the formyl group (–CHO), regulates $tRNA_i^{Met}$ biology across three successive layers of molecular organization—protein, RNA, and DNA (Figure 1). Although these formyl-dependent mechanisms were discovered in very different contexts and are installed by biochemically unrelated pathways (Table 1), they converge on the regulation of translation, especially on efficient initiation and elongation during protein synthesis. In this opinion article, it is proposed that these mechanisms constitute a **formyl axis**, a multilayer regulatory system unified by shared chemical logic and common metabolic dependence, notably on OCM and mitochondrial activity. Via this input, the formyl axis may function as a rheostat that couples cellular metabolic state to translation.

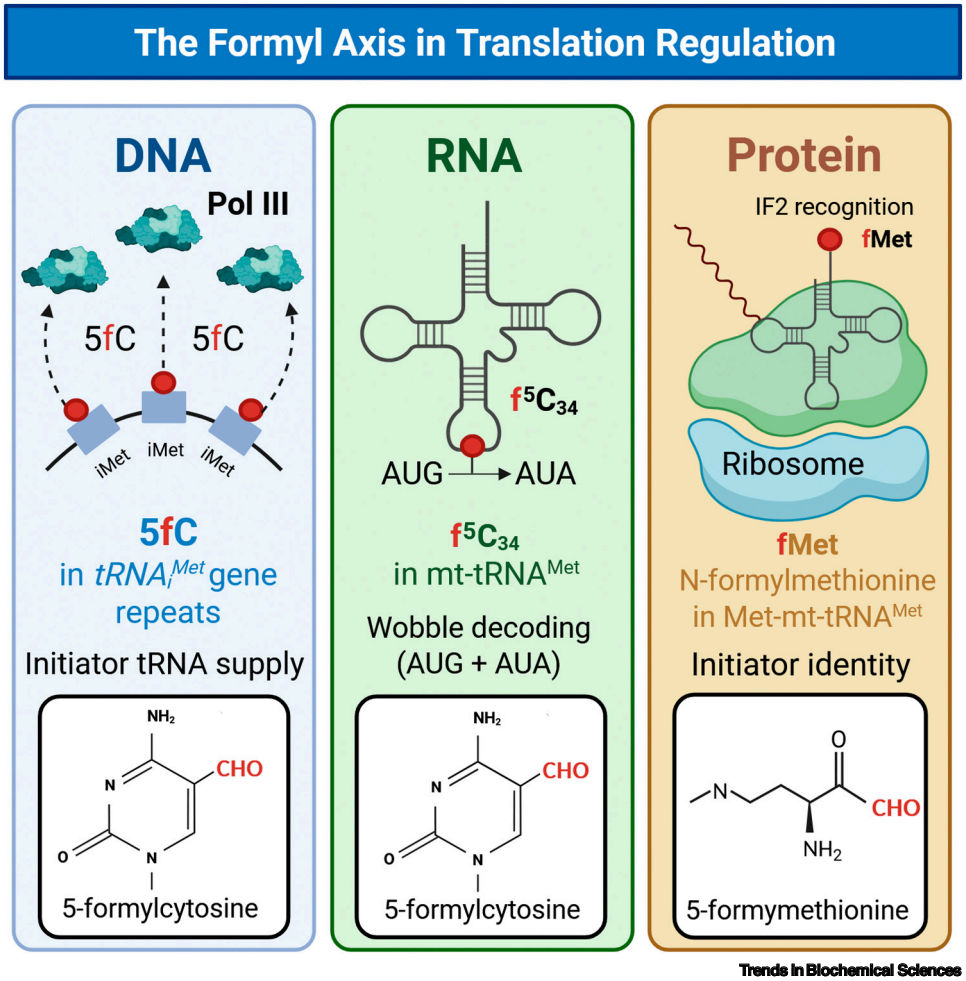


Figure 1. The formyl axis in translation initiation. Formyl chemistry regulates initiator methionine biology at three molecular levels. In the DNA layer, 5-formylcytosine (5fC) accumulates at initiator methionine tRNA methionine ($tRNA_i^{Met}$) gene tandem repeats, where it promotes the recruitment of RNA polymerase III (Pol III) and increases the initiator transfer RNA supply. In the RNA layer, 5-formylcytidine at wobble position 34 (f^5C_{34}) in mitochondrial methionine tRNA methionine ($mt\text{-}tRNA^{Met}$) expands methionine decoding to include both AUG and AUA codons. In the protein layer, N-formylmethionine (fMet) on initiator methionyl-tRNA promotes recognition by initiation factor 2 (IF2), thereby specifying initiator identity during translation initiation. Thus, the same formyl group is used in three distinct molecular contexts. In DNA, it epigenetically activates initiator *tRNA* genes; in RNA, it serves wobble decoding; and at the protein synthesis level, it allows initiator discrimination. Together, these three layers define a formyl axis that converges on initiator methionine tRNA and the regulation of translation. Red balls and lettering (f, CHO) indicate the formyl group. Figure created using BioRender (https://BioRender.com).

Glossary

$fMet\text{-}mt\text{-}tRNA^{fMet}$: the formylated mitochondrial initiator methionyl-tRNA. MTFMT formylates a fraction of $Met\text{-}tRNA^{Met}$, promoting IF2 recognition and initiation of translation of the 13 mitochondrially encoded proteins.

fMet/N-degron pathway: a stress-induced post-translational pathway that eliminates completed proteins retaining N-terminal formylmethionine.

fMet-ribosome-associated quality control pathway: a co-translational ribosome quality control pathway induced by stress and triggered by fMet-bearing nascent chains during elongation.

Formyl axis: the proposed regulatory framework in which three formyl-based modifications (at the DNA, RNA, and protein levels) coordinately regulate $tRNA_i^{Met}$ and translation through co-dependence on OCM and mitochondrial activity.

Initiator methionine tRNA ($tRNA_i^{Met}$): also known as iMet; a specialized cytosolic tRNA responsible for recognizing the start codon and initiating translation in the cytoplasm; it is distinct from elongator tRNAs in structure and function, as well as $mt\text{-}tRNA^{Met}$, and is rate-limiting for global translation initiation rates.

α-ketoglutarate (αKG): a metabolic intermediate generated notably by the TCA cycle and an essential co-substrate for αKG-dependent dioxygenases.

α-ketoglutarate (αKG)-dependent dioxygenase: a large enzyme superfamily, including TET proteins and ALKBH1, that use O_2, Fe^{2+}, and αKG to oxidize DNA or RNA bases, thereby linking epigenetic modification to metabolism.

5,10-methylenetetrahydrofolate: a central folate intermediate at the branch point between reductive flux toward SAM and oxidative flux toward 10-formyl-THF, thereby influencing methyl-donor and formyl-donor supply.

Mitochondrial tRNA methionine ($mt\text{-}tRNA^{Met}$): the single metazoan mitochondrial methionine tRNA, serving both initiator and elongator functions. After aminoacylation, a fraction is formylated by MTFMT to generate $fMet\text{-}mt\text{-}tRNA^{fMet}$ for translation initiation.

One-carbon metabolism (OCM): a metabolic network centered on folate and methionine cycling that distributes single-carbon units for nucleotide synthesis, methylation reactions, and

Three layers of the formyl axis

The formyl axis comprises three mechanistically distinct layers, each deploying a formyl modification to regulate a different aspect of $tRNA_i^{Met}$ biology (Figure 1 and Table 1).

The protein layer

Within the formyl axis, the protein layer is unique because its formyl mark is installed on Met-charged initiator tRNA, yet it acts on protein synthesis and protein fate. By contrast, DNA and RNA marks act on the modified nucleic acids themselves.

In bacteria, chloroplasts, and mitochondria, initiation uses N-formylmethionine (fMet), generated when the formyltransferases methionyl-tRNA formyltransferase (FMT, in bacteria and chloroplasts) and mitochondrial methionyl-tRNA formyltransferase (MTFMT) transfer a formyl group from 10-formyl-tetrahydrofolate (10-formyl-THF) to the methionylated initiator tRNA. This product is commonly termed fMet-$tRNA^{fMet}$ in bacteria and chloroplasts, whereas in mammalian mitochondria it is designated as fMet-mt-$tRNA^{Met}$ because the single mt-$tRNA^{Met}$ serves both initiator and elongator roles [11,12]. This modified tRNA initiates translation, including in mammalian mitochondria. The formyl group is the biochemical signal that enables recognition by initiation factor 2 (IF2) while preventing the tRNA from binding to elongation factor Tu (EF-Tu), thereby enforcing start-site fidelity (Box 1).

This compartmental separation in mitochondria is not absolute. Following cold stress and starvation, eukaryotic cells can also generate N-terminally formylated proteins in the cytosol, and fMet has emerged as a conditional proteostasis signal in which the formyl group is installed pre-translationally on the cytosolic methionyl-$tRNA_i^{Met}$ [16]. Two mechanistically distinct pathways subsequently act on this signal. Co-translationally, the **fMet-ribosome-associated quality control** pathway arrests translation of N-terminal (Nt)-fMet-bearing nascent chains, routing the product toward protein degradation [17]. Post-translationally, the **fMet/N-degron pathway**

formyl transfer; it integrates inputs from, for example, serine, glycine, folate, vitamin B12, and the TCA cycle.
S-adenosylmethionine (SAM): the major cellular methyl donor, synthesized from methionine and ATP.
Tricarboxylic acid (TCA): a central metabolic pathway that generates energy and biosynthetic intermediates.
Zygotic genome activation (ZGA): the developmental transition during which the embryonic genome becomes transcriptionally active; it is accompanied by epigenetic remodeling and increased translational demand.

Table 1. Formyl axis across three regulatory layers

Feature	DNA layer	RNA layer	Protein layer
Regulatory level	Epigenetic	Epitranscriptomic	Translational
Cellular context	Nuclear *tRNA* gene regulation	Mitochondrial decoding by tRNA	Bacteria, chloroplast, and mitochondria translation initiation
Substrate	Nuclear $tRNA_i^{Met}$ gene clusters	mt-$tRNA^{Met}$	Met-mt-$tRNA^{Met}$
Formyl modification	5-formyl-2′-deoxycytosine (5fC) at CpG dinucleotides	f^5C_{34}	fMet
Installation logic and enzymes involved	5mC oxidation by TET enzymes	NSUN3-dependent m^5C_{34} formation followed by ALKBH1 oxidation to f^5C_{34}	Direct formyl transfer by FMT (bacteria) and MTFMT (mammals)
Key cofactors/donors	SAM (precursor methylation), Fe^{2+}, αKG, and O_2	Same as DNA	10-formyl-THF
Eraser/turnover mechanisms	TDG and BER	None known (set by writing + tRNA turnover)	Mitochondrial peptide deformylase (PDF)
Mechanistic role in initiator methionine regulation and translation initiation	Promotes Pol III transcription at $tRNA_i^{Met}$ loci, ensuring sufficient initiator tRNA supply	Expands mt-$tRNA^{Met}$ wobble decoding, enabling methionine reading of both AUG and AUA	Promotes recognition by IF2 and disfavors elongation factor usage, thereby establishing initiator aminoacyl-tRNA identity
Most direct OCM input	SAM (via serine → 5,10-methylene-THF → MTHFR → methionine → SAM)	Same as DNA	10-formyl-THF (via serine → 5,10-methylene-THF → MTHFD2)
Mitochondrial input	αKG for TET dioxygenase; OCM for SAM supply	αKG for ALKBH1 dioxygenase; OCM for SAM supply	Matrix NAD^+/NADH ratio affecting MTHFD2 flux

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Box 1. The protein layer—fMet in translation initiation

fMet is the universal initiating amino acid in bacteria and is also typically used to initiate translation in mitochondria and chloroplasts, where it is generated by FMT (in bacteria) and MTFMT (in mitochondria) using 10-formyl-THF as the formyl donor [11,12]. 10-formyl-THF is produced from 5,10-methylene-THF by MTHFD2 in the oxidative branch of the folate cycle, placing fMet synthesis in direct metabolic connection with one-carbon flux. In bacteria, FMT acts selectively on the dedicated initiator $tRNA^{Met}$, recognizing structural identity elements concentrated in its acceptor stem. In mitochondria, MTFMT acts on the single mt-$tRNA^{Met}$ that serves both initiator and elongator functions.

Biochemically, formylation helps channel the charged initiator tRNA into initiation rather than elongation. Adding the formyl group reshapes the aminoacyl end of the tRNA in a way that favors IF2-dependent initiation complex formation and disfavors EF-Tu binding [11,12]. This single covalent modification thus enforces the biochemical distinction between initiation and elongation, without requiring a separate amino acid or a fundamentally different charging mechanism. The formyl group on initiator methionine is therefore the oldest and most direct molecular link between formyl chemistry and translation initiation.

The physiological importance of formylation for translation initiation is demonstrated by the disease consequences of its loss. Mutations in human MTFMT cause Leigh syndrome and combined OXPHOS deficiency, with patient cells showing severely reduced fMet-tRNA levels and impaired mitochondrial protein synthesis, which is rescued by MTFMT re-expression [13]. Importantly, fMet-tRNA synthesis is not constitutively saturated but is dynamically coupled to one-carbon flux. Mitochondrial serine catabolism by SHMT2 is required for maintaining formylmethionine levels and proper mitochondrial translation initiation [14]. When SHMT2 is depleted, 10-formyl-THF availability declines and fMet-tRNA levels drop, directly impairing mitochondrial translation. SHMT2 is frequently upregulated in rapidly proliferating cells and in many cancers, consistent with the increased demand for mitochondrial OCM in these settings [15]. This supports the idea that the protein formyl layer is regulated by one-carbon flux, such that metabolic upregulation of the SHMT2-dependent folate pathway expands the available pool of 10-formyl-THF to drive increased fMet-tRNA synthesis in response to high translational demand.

targets the completed Nt-fMet protein for proteasomal degradation in mammals via the E3 ubiquitin ligase TRIM52 (Tripartite Motif Containing 52) [16]. Both pathways therefore couple the same pre-translationally installed formyl mark to translational regulation—one during synthesis, the other shortly after completion—and both are negative in effect, promoting arrest and turnover rather than productive output. Thus, fMet functions as a metabolically responsive regulator of translation more broadly, not only licensing faithful initiation but also flagging aberrant or stress-associated products for arrest and disposal. These protein-level functions—initiation fidelity, quality control, and degradation—define this as the formyl–protein layer.

The RNA layer

The RNA layer adds a second mitochondrial use of formyl chemistry (Box 2). The single mitochondrial $tRNA^{Met}$ (**mt-$tRNA^{Met}$**) serves as both the initiator and the elongator, decoding AUG and AUA codons as methionine. This is enabled by 5-formylcytidine at wobble position 34 (f^5C_{34}), installed by NOP2/Sun-RNA-Methyltransferase 3 (NSUN3) and AlkB Homolog 1, Histone H2A Dioxygenase (ALKBH1) [20–22]. Thus, one mt-$tRNA^{Met}$ carries two formyl-dependent features: anticodon f^5C_{34} and fMet. Together, the two formyl units support translation initiation and elongation (via AUA decoding), respectively (Boxes 1 and 2). Because mammalian f^5C is essentially limited to mt-$tRNA^{Met}$ [27], this dual use suggests a broader organizing principle in $tRNA_i^{Met}$ biology. The DNA layer further extends this pattern.

The DNA layer

The DNA layer of the formyl axis is the most recently described branch. We found that 5-formylcytosine (5fC) accumulates at polymerase III (Pol III)-transcribed *tRNA* gene tandem repeats during **zygotic genome activation** (**ZGA**) in *Xenopus* (frog) embryos [28]. 5fC in DNA is generated by ten-eleven translocation (TET)-mediated oxidation of 5-methylcytosine (5mC), and the affected Pol III target loci prominently include $tRNA_i^{Met}$ genes (i.e., those coding for cytosolic $tRNA_i^{Met}$). At these loci, 5fC promotes Pol III recruitment, increasing their transcription

Box 2. The mitochondrial RNA layer—f^5C in *tRNA*^{Met}

Human mitochondria encode a highly reduced set of 22 tRNAs, including a single $tRNA^{Met}$ that must function in both initiation and elongation [18]. In vertebrate mitochondria, AUA is read as methionine rather than isoleucine. As a result, mt-$tRNA^{Met}$ has to accommodate both conventional AUG decoding and noncanonical reading of AUA. An unmodified cytidine at wobble position 34 cannot pair efficiently with adenosine, and unmodified mt-$tRNA^{Met}$ transcripts decode AUA poorly in reconstituted translation systems [19].

The solution is f^5C at position 34, installed through a two-step enzymatic pathway that recapitulates the methylation-then-oxidation sequence characteristic of the DNA layer. NSUN3 first methylates C34 to generate m^5C34. NSUN3 belongs to the conserved NSUN family of RNA methyltransferases and acts on $tRNA^{Met}$ as its prominent substrate. Second, the αKG-, Fe^{2+}-, and O_2-dependent dioxygenase ALKBH1 oxidizes the methyl group to a formyl group, yielding f^5C_{34}, likely through a hm^5C_{34} intermediate [20–22]. ALKBH1 belongs to the same AlkB (Alkylating agent-sensitive mutant B) superfamily of dioxygenases as the TET enzymes and shares their co-substrate dependence. Both ALKBH1 and NSUN3 localize to mitochondria, and the loss of either abolishes f^5C_{34} and impairs mitochondrial translation.

The formyl group at C34 permits more favorable pairing with adenosine at the wobble position [19]. This expanded decoding capacity is important because, without f^5C_{34}, the single mt-$tRNA^{Met}$ cannot read the full complement of mitochondrial methionine codons. This compromises not only elongation at internal AUA positions but also the overall efficiency of mitochondrial translation, as normally initiated ribosomes stall at internal AUA codons.

The physiological importance of f^5C_{34} for mitochondrial translation is underscored by the disease consequences of its deficiency. Loss-of-function variants in NSUN3 and ALKBH1 are associated with combined OXPHOS deficiency and mitochondrial encephalopathy, placing f^5C_{34} within the growing class of 'mitochondrial tRNA modopathies' [23]. In mouse models, NSUN3 deletion is embryonically lethal, with impaired mitochondrial translation and respiratory chain assembly [24], demonstrating that f^5C_{34} is essential for mitochondrial protein synthesis. f^5C_{34} installation is not static but is dynamically responsive to the mitochondrial metabolic state [25,26]. Taken together, the RNA formyl layer ensures that mt-$tRNA^{Met}$ maintains effective mitochondrial translation and function in response to high-energy demands.

and the supply of the $tRNA_i^{Met}$ pool (Box 3). At present, this DNA-layer evidence is strongest in *Xenopus* ZGA. Whether analogous regulation occurs in other organisms or other contexts of translation control remains an open question.

Box 3. The DNA layer—5fC at initiator *tRNA* gene clusters

5mC is the canonical DNA epigenetic mark in vertebrates and is mostly associated with gene silencing. 5mC is a dynamic epigenetic mark that can be removed in a locus-specific manner by active DNA demethylation, a process at the center of which are the TET1/2/3 dioxygenases [29]. This family of αKG-, Fe^{2+}-, and O_2-dependent enzymes iteratively oxidizes 5mC through a stepwise pathway: 5mC → 5-hydroxymethylcytosine → 5fC → 5-carboxylcytosine (5caC). 5fC and 5caC are excised by TDG, followed by BER to restore unmodified cytosine. While TET proteins also have noncatalytic functions, during development their enzymatic activity is essential for embryonic reprogramming through active DNA demethylation [30]. In cancer, TET mutations impair this demethylation capacity, leading to aberrant gene hypermethylation and gene silencing [29].

While 5fC is primarily a DNA demethylation intermediate, it can also accumulate at enhancers and promoters [31–33]. Indeed, we recently showed that 5fC functions as an instructive activating epigenetic mark in its own right for Pol III transcription during ZGA [28]. ZGA marks a major transition in embryogenesis and coincides with an acute demand for *de novo* protein synthesis. Accordingly, Pol III targets, including *tRNA* genes, are strongly upregulated to scale translation initiation with embryonic growth. Genome-wide mapping in *Xenopus* revealed 5fC enrichment at *tRNA* gene clusters, which are tandem repeats that span tens to hundreds of kilobases and are expressed first during oogenesis and again at ZGA [28,34]. One well-characterized cluster contains 42 tandem copies of an approxiamtely 3-kb repeat unit encoding seven *tRNA* genes, including two $tRNA_i^{Met}$ genes that are prominently enriched for 5fC.

tRNA genes are unusually well positioned to respond directly to DNA modifications such as 5fC. Unlike Pol II-transcribed genes, they contain internal promoter elements within the transcribed region. Their regulatory A- and B-boxes are recognized by TFIIIC (Transcription Factor IIIC), which in turn recruits Pol III for transcription initiation. Because this promoter architecture lies within the gene body, DNA modifications at *tRNA* loci can directly affect Pol III engagement. Whether 5fC promotes the recruitment of TFIIIC and Pol III by altering DNA structure, recruiting specific reader proteins, or repelling inhibitory factors is unknown.

In conclusion, during ZGA, when translation initiation capacity must expand sharply, 5fC promotes transcription of $tRNA_i^{Met}$ genes. The DNA formyl layer, therefore, controls initiator tRNA supply through a TET-dependent, OCM-coupled mechanism that shares dioxygenase cofactors with the RNA formyl layer.

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One chemical motif, three molecular contexts

As summarized in Table 1, these three formyl modifications are chemically analogous: all are aldehydes installed on or near the initiator methionine residue, but they are synthesized by entirely different enzyme families, act on different substrates (methionyl-tRNA, cytosine in RNA vs. DNA), and operate in different cellular compartments (mitochondrial matrix vs. nucleus). There is no evidence that one modification directs the installation of another. What, then, may be the underlying biological rationale for this formyl axis?

The formyl group confers electrophilicity, hydrogen-bonding capacity, compactness, and the ability to modulate the electronic character of adjacent atoms, which may make it unusually versatile as a regulatory element. Yet in each layer, these properties are deployed through a different mechanism to solve a different problem. At the protein level, the N-formyl group blocks the α-amino group of initiating methionine, creating a steric and electronic surface recognized by IF2 and incompatible with EF-Tu binding [35]. At the RNA level, the C5-formyl group on cytidine 34 alters the tautomeric and hydrogen-bonding properties of the pyrimidine ring, broadening wobble pairing geometry to accommodate adenosine at the third codon position [19]. At the DNA level, 5fC may increase local DNA flexibility and bendability, facilitating TFIIIC/Pol III recruitment to *tRNA* genes [36].

Since the same chemical group is deployed in mechanistically distinct ways at each layer, what unites these divergent functions is not a shared installation mechanism or mode of downstream signaling. Instead, I argue that the formyl axis reflects a shared metabolic logic operating upstream through two convergent metabolic inputs.

First, all three layers are linked to OCM, either through direct transfer from 10-formyl-THF in the protein layer or through oxidation of SAM-derived methyl groups in the DNA and RNA layers (Figure 2). Serine-dependent folate flux provides a major route into this network. This renders the axis intrinsically sensitive to folate, serine, and methionine status. Second, the efficiency of formyl-group installation is governed by the mitochondrial metabolic state. At the DNA and RNA layers, **α-ketoglutarate (αKG)-dependent dioxygenase** activity controls the oxidation step. At the protein layer, mitochondrial matrix redox balance (NAD^+/NADH) controls the necessary folate flux. This dependence on OCM and mitochondrial activity is what makes the formyl axis a metabolically integrated system.

The formyl axis as a predicted metabolic rheostat

The central hypothesis here is that the three arms of the formyl axis are not merely chemically analogous but metabolically linked by two convergent upstream dependencies, OCM and mitochondrial metabolic state, that together allow them to function as a metabolic rheostat for translation initiation (Figure 2). OCM supplies the formyl carbon at every layer through 10-formyl-THF production in the protein layer and SAM-dependent methylation in the DNA and RNA layers. Mitochondrial metabolic state, in turn, modulates whether formylation proceeds by controlling matrix NAD^+-dependent one-carbon flux in the protein layer and αKG-dependent dioxygenase activity in the DNA and RNA layers. For the nuclear DNA layer, αKG availability may reflect not only mitochondrial **tricarboxylic acid (TCA) cycle** activity but also other αKG sources from glutaminolysis, cytosolic reductive metabolism, nutrient status, and metabolite transport. Moreover, a substantial part of OCM is itself mitochondrial and contributes to matrix redox balance and formyl precursor supply. Together, these dependencies position the formyl axis as a metabolically responsive system organized around one-carbon supply and mitochondrial activity (Figure 2).

One-carbon flux through the folate and methionine network

The three branches of the formyl axis are linked to OCM either through direct transfer from 10-formyl-THF at the protein layer or through oxidation of SAM-derived methyl groups at the DNA

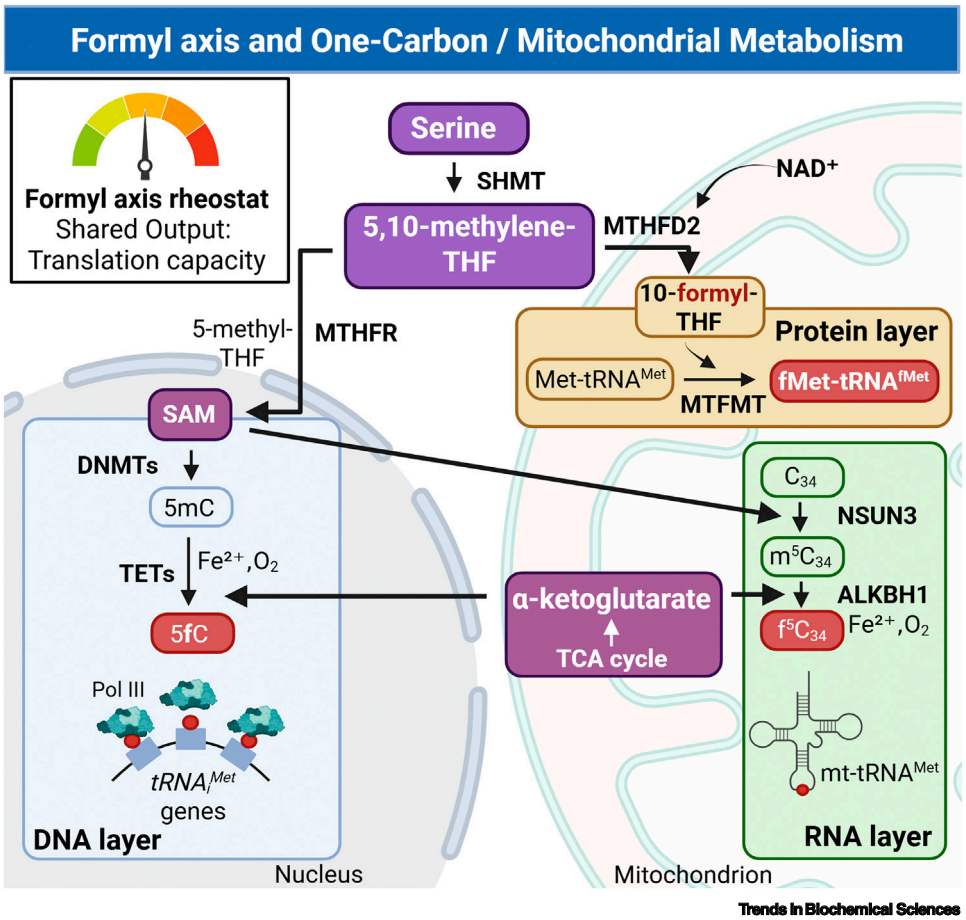


Figure 2. Formyl axis and one-carbon and mitochondrial metabolism. A simplified metabolic scheme illustrates two major metabolic inputs that regulate the formyl axis, omitting many intermediate metabolites, cofactors, byproducts, transport steps, and reversible reactions for clarity. The formate shuttle, connecting the mitochondrial and cytoplasmic folate pools, is also omitted, as are the cytoplasmic stress-related fMet/N- and fMet-RQC pathways. Serine enters the folate cycle through serine hydroxymethyltransferase (SHMT), generating 5,10-methylene-tetrahydrofolate (5,10-methylene-THF) as a central branch point metabolite. Note that while serine-dependent folate flux provides a major route into this network, glycine, formate, histidine, choline-derived metabolism, and methionine/S-adenosylmethionine (SAM) salvage can also contribute one-carbon units in a context-dependent manner. The reductive branch, via methylenetetrahydrofolate reductase (MTHFR), supports synthesis of methionine and SAM, which supplies the methylation precursor steps for formyl-group installation in the DNA and RNA layers. In the DNA layer, DNA methyltransferases (DNMTs) generate 5-methylcytosine (5mC), which is oxidized by ten-eleven translocation (TET) dioxygenases to 5fC at $tRNA_i^{Met}$ tandem-repeat genes. In the RNA layer, the NOP2/Sun-RNA-Methyltransferase 3 (NSUN3) and the AlkB Homolog 1, Histone H2A Dioxygenase (ALKBH1) generate f^5C_{34} in mt-$tRNA^{Met}$. The oxidative branch, via methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), generates 10-formyl-tetrahydrofolate (10-formyl-THF), the direct formyl donor used by mitochondrial methionyl-tRNA formyltransferase (MTFMT) in the protein layer to produce fMet-mt-$tRNA^{Met}$. The DNA and RNA layers also depend on αKG, oxygen, and Fe^{2+}, linking their oxidative chemistry to mitochondrial and oxidative metabolic states. In the nuclear DNA layer, αKG availability may reflect TCA cycle activity in some contexts but can also be shaped by glutaminolysis, cytosolic metabolism, nutrient status, and metabolite transport. Together, these shared inputs suggest that the formyl axis may act as a metabolic rheostat for translational regulation. Red balls and lettering indicate formyl groups or formylated biomolecules. Nucleus and mitochondrion are not drawn to scale. Figure created using BioRender (https://BioRender.com). Pol III: polymerase III; TCA: tricarboxylic acid.

and RNA layers. In the simplified scheme shown in Figure 2, **5,10-methylenetetrahydrofolate** (5,10-methylene-THF) is depicted as a central folate branchpoint. Reduction by MTHFR yields 5-methyl-THF, which regenerates methionine and, ultimately, SAM, the methyl donor consumed by

DNA methyltransferase (DNMT; DNA layer) and RNA methyltransferase NSUN3 (RNA layer) in the methylation steps that precede formyl group installation. At the same time, oxidation by methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) yields 10-formyl-THF, the direct formyl donor for MTFMT at the protein layer [4]. The DNA and RNA layers thus occupy the methyl/SAM-dependent side of this network, while the protein layer directly uses the 10-formyl-THF branch (Figure 2).

A major convergence occurs at serine. As a principal one-carbon donor to the folate cycle via serine hydroxymethyltransferase (SHMT), serine feeds the 5,10-methylene-THF pool and provides a key entry point into the model. However, although not shown for simplicity in Figure 2, the folate–methionine network is not serine-exclusive. Glycine, formate, histidine, choline-derived metabolism, and methionine/SAM cycling can also contribute one-carbon units depending on the organism, tissue, nutrient state, and compartment. Thus, serine provides a central, but not exclusive, route into the one-carbon substrate logic of the formyl axis.

While the availability of one-carbon units is necessary, it is not sufficient for formylation. Whether the methyl precursors at the DNA and RNA layers are converted into formyl products and whether 10-formyl-THF is produced at a sufficient rate in the protein layer also depend on a second upstream input, namely, the mitochondrial and oxidative metabolic state.

Mitochondria, α-KG, and redox gating

The methylation-then-oxidation pathways of the DNA and RNA layers share additional metabolic dependencies. Both, TET enzymes (DNA layer) and ALKBH1 (RNA layer), are Fe^{2+} and oxygen-dependent dioxygenases that require αKG as a co-substrate [37]. For the nuclear DNA layer, αKG availability may reflect mitochondrial TCA cycle activity in most contexts, but it can also be shaped by glutaminolysis, cytosolic reductive metabolism, nutrient status, and metabolite transport. TET and ALKBH1 dioxygenases are competitively inhibited by succinate, fumarate, and α-hydroxyglutarate, oncometabolites that accumulate when TCA cycle or electron transport chain function is impaired.

The protein layer is coupled to mitochondrial activity through a different mechanism. MTFMT does not require αKG, Fe^{2+}, or O_2 directly, but its substrate, 10-formyl-THF, is generated in the mitochondrial matrix by MTHFD2, an NAD^+/$NADP^+$-dependent enzyme whose activity is linked to the matrix redox balance. Under hypoxia or respiratory chain dysfunction, NADH accumulates, NAD^+ availability may decline, and MTHFD2 flux is expected to decrease, reducing 10-formyl-THF production and constraining fMet-tRNA synthesis.

Thus, the DNA and RNA layers are coupled to oxygen- and αKG-dependent dioxygenase chemistry, whereas the protein layer is coupled more indirectly to mitochondrial redox-supported one-carbon flux. All three layers are therefore connected to mitochondrial and oxidative metabolism, but through distinct mechanisms rather than a single shared cofactor dependency. Thus, OCM and mitochondrial activity should not be viewed as independent parallel inputs to the formyl axis. Instead, mitochondrial OCM contributes both to precursor supply and to matrix redox balance. The formyl axis is therefore best described as a system with two major, functionally distinguishable but biochemically overlapping inputs—one-carbon substrate supply and mitochondrial/oxidative gating.

Under growth-permissive conditions, when both serine/folate/SAM sustain one-carbon flux and αKG/oxygen/iron/matrix NAD^+ support mitochondrial input, all three arms of the formyl axis are predicted to be favored ($tRNA_i^{Met}$ transcription, full mt-$tRNA^{Met}$ decoding capacity, fMet-mt-

tRNA^{Met} synthesis). Conversely, constraint on either metabolic input is predicted to dampen one or more layers, depending on buffering, compartment, and metabolic context. Such coordination would not require dedicated crosstalk but could arise from shared upstream input. Hence, the formyl axis is proposed as a metabolic rheostat that senses one-carbon biosynthetic capacity and mitochondrial/oxidative competence, while remaining open to additional nutrient-specific, compartment-specific, and context-specific inputs.

Flux and turnover of formyl marks

The level of a formyl mark depends not only on its rate of installation but also on removal and, hence, turnover. Each arm of the formyl axis should therefore be viewed as a turnover system. In the protein layer, MTFMT installs the formyl group, whereas mitochondrial peptide deformylase removes N-terminal formyl groups from nascent mitochondrial proteins [16]. In the DNA layer, TET enzymes generate 5fC, whereas thymine DNA glycosylase (TDG) and base excision repair (BER) remove 5fC during active DNA demethylation [30]. In the RNA layer, no dedicated f^{5}C$_{34}$ eraser is known, and hence its steady-state levels likely reflect the balance between writer activity, mitochondrial tRNA biogenesis, and tRNA turnover. This flux-and-turnover view may explain why some perturbations reveal buffering or threshold behavior rather than simple graded tuning.

Metabolic control of Met-tRNA^{Met} formylation

Although simultaneous co-regulation of all three formyl layers remains untested, metabolic regulation of each individual arm is well supported. Key evidence that OCM controls the protein-layer formyl axis comes from a study showing that mitochondrial serine catabolism by SHMT2 is required to maintain **fMet-mt-tRNA^{fMet}** and proper mitochondrial translation initiation [14]. SHMT2 deficiency reduced fMet-mt-tRNA^{fMet}, shifted the initiator pool toward unformylated Met-mt-tRNA^{fMet}, and these defects were rescued by formate. The phenotype also overlapped with that of MTFMT deficiency, supporting the dependence of the protein formyl layer on mitochondrial one-carbon flux. Genetic analysis further showed that the relevant source is the mitochondrial, not cytoplasmic, one-carbon pool, with 10-formyl-THF generated via MTHFD2/2L from SHMT2-derived one-carbon units. Thus, fMet-mt-tRNA^{fMet} synthesis is biochemically dependent on mitochondrial one-carbon flux. However, these effects required near-complete SHMT2 loss, whereas minimal residual SHMT2 expression preserved fMet-mt-tRNA^{fMet}, indicating substantial buffering and threshold behavior. Whether physiological variation in one-carbon flux dynamically modulates fMet-mt-tRNA^{fMet} therefore remains unclear.

In yeast, it has been shown that serine recycled through autophagy supplies the fMet-mt-tRNA^{fMet} pool, thereby supporting mitochondrial protein synthesis and adaptive metabolic rewiring under nutrient stress [38]. The physiological importance of this pathway is further underscored by pathogenic variants of the formylating enzyme *MTFMT* in humans, which cause Leigh syndrome and combined oxidative phosphorylation (OXPHOS) deficiency [13]. In addition, mtDNA haplogroup-defining variants influence circulating fMet levels, and higher blood fMet is associated with the risk of multiple late-onset diseases [39], while in mice, a high-fat-diet-induces upregulation of *Mtfmt* [40]. Overall, the evidence supports the metabolic dependence of the RNA–protein layer of the formyl axis on one-carbon flux, although it remains an open question whether this behaves as a graded rheostat.

Metabolic control of f^{5}C formation in mt-tRNA^{Met}

The RNA-layer formyl axis, namely the installation of f^{5}C$_{34}$ of mt-tRNA^{Met}, is directly coupled to metabolic state through the cofactor dependencies of its biosynthetic pathway. NSUN3 uses

SAM to generate the m^5C_{34} precursor, whereas ALKBH1 is an αKG-, Fe^{2+}-, and O_2-dependent dioxygenase that oxidizes m^5C_{34} to f^5C_{34}, thereby linking the pathway to oxygen availability and αKG-dependent mitochondrial metabolism [20,41]. Indeed, a recent study concludes that these m5C/f5C C34 modifications function as 'sensors of cellular energy requirements' [25], gating the metabolic plasticity between glycolysis and OXPHOS. Cancer cells lacking m^5C/f^5C C34 actively rebalance their energy production toward glycolysis, whereas cells capable of dynamically re-establishing high modification levels, such as metastasis-initiating cells, retain the capacity to switch back to OXPHOS to drive invasion. Because pharmacological inhibition of mitochondrial translation phenocopies NSUN3 loss, the authors of the study conclude that f^5C_{34}-dependent translation directly adapts mitochondrial function to the cellular energy state.

Concordantly, a recent preprint reports that f^5C and 5-hydroxymethylcytidine (hm^5C) levels decline under mitochondrial stress induced by mitochondrial poisons, without changes in NSUN3 or ALKBH1 protein abundance, suggesting regulation via enzymatic cofactor availability [26]. This architecture may create a feed-forward loop [3]: active mitochondrial metabolism supplies the αKG that ALKBH1 requires to install f^5C_{34}, which in turn enables the translation of the respiratory chain protein subunits needed to sustain the TCA cycle.

Other studies on *SHMT2*-deficient cells show mitochondrial ribosomal stalling due to defective wobble-base methylation (taurinomethyluridine) of certain mt-tRNAs [42,43], but N-terminal fMet peptides and $tRNA^{Met}$ cytosine methylation were unchanged. Also, mitoribosome profiling did not support defective AUA decoding by f^5-$tRNA^{Met}$. Nevertheless, these studies support the broader idea that OCM regulates mitochondrial translation through 5,10-methylene-THF-dependent RNA modification. They also indicate that this regulation may manifest in a cell-context-specific manner through modifications of tRNAs beyond mt-$tRNA^{Met}$.

Metabolic control of 5fC formation in $tRNA^{iMet}$ DNA

As described in Box 3, 5fC accumulates at initiator $tRNA_i^{Met}$ gene clusters during ZGA, where it promotes Pol III recruitment and $tRNA_i^{Met}$ transcription. The fact that this epigenetic activation occurs precisely when the embryo transitions from maternal to zygotic gene expression, a phase demanding a massive expansion in translational capacity, suggests that 5fC-mediated production of initiator tRNA is coupled to the cell's translational needs. But is it also coupled to its metabolic state?

Independent evidence strongly suggests this and reveals the dual-input architecture of the DNA layer. On the substrate side, DNA methylation by DNMTs consumes SAM, and SAM availability is sensitive to methionine status, folate levels, and serine-dependent one-carbon flux (Figure 2). For example, methionine restriction modulates hepatic DNA methylation through altered SAH levels [44], serine metabolism drives SAM-dependent DNA hypermethylation downstream of mTOR in LKB1-deficient cancers [45], and folate deficiency promotes genome-wide changes in DNA methylation and hydroxymethylation [46].

On the dioxygenase side, TET enzymes are among the best-characterized metabolic sensors in epigenetics. TET activity responds to intracellular αKG levels, which directly sustain TET-dependent demethylation in embryonic stem cells [47], while tumor hypoxia reduces TET activity and causes promoter hypermethylation [48].

Whether these metabolic inputs specifically regulate 5fC deposition at $tRNA_i^{Met}$ gene clusters has not been tested. However, because the dioxygenase step is the rate-limiting and physiologically dynamic step in the 5mC → 5fC conversion, the DNA layer is predicted to be primarily responsive

to the mitochondrial metabolic state, with one-carbon/SAM flux providing the necessary but less dynamically regulated substrate.

Together, the data support a rheostat model wherein OCM and mitochondrial activity scale protein biosynthesis to metabolic capacity. The model makes testable predictions (see Outstanding questions).

Concluding remarks

The formyl axis highlights how one small chemical group has been redeployed across protein, RNA, and DNA layers to regulate different aspects of $tRNA_i^{Met}$ biology. Together, these layers suggest a metabolic rheostat that integrates OCM and mitochondrial activity. Evolutionarily, this axis was likely assembled by convergent redeployment rather than co-invention. The fMet-tRNA layer is ancient and bacterial in origin; the RNA layer represents a later mitochondrial adaptation, and the DNA layer appears most recent. Its evolutionary reconstruction may help explain why formyl chemistry was repeatedly selected at the interface of metabolism and translation. The next step, though analytically challenging, is simultaneous measurement of fMet-mt-$tRNA^{Met}$, mt-$tRNA^{Met}$ f5C34, and $tRNA^{iMet}$-locus 5fC under controlled perturbations of folate, serine, methionine, αKG, oxygen, and respiratory chain activity. It should reveal whether the axis behaves as a graded rheostat, a thresholded switch, or a set of independently buffered modules. Therapeutically, the framework may be most relevant where OCM and mitochondrial state are already perturbed, including neurometabolic disease (frequently associated with mitochondrial defects), Isocitrat-Dehydrogenase (IDH)-mutant cancer, and tumors dependent on SHMT2 or MTHFD2. More broadly, the formyl axis may provide a template for identifying other metabolism-linked regulatory systems built from recurring chemical motifs.

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Declaration of interests

The author declares no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used ChatGPT and Anthropic Claude for literature review and language editing. After using these tools, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Outstanding questions

Do the three formyl modifications respond 'coordinately' to perturbation of OCM and mitochondrial oxidative state, and does the system behave as a graded rheostat or as a thresholded switch? Simultaneous measurement of fMet-mt-$tRNA^{fMet}$, f^5C_{34}, and 5fC at $tRNA_i^{Met}$ gene clusters under folate/serine/methionine and mitochondrial manipulation would directly test the hypothesis advanced here. Although 5fC at $tRNA_i^{Met}$ genes has so far been shown only in *Xenopus*, potential co-regulation of fMet-tRNA levels and f^5C34 in mt-tRNAMet could be analyzed in mammalian cell lines.

Because SHMT2 supplies mitochondrial one-carbon units feeding all three formyl layers, does its perturbation reveal shared substrate dependence—and is this dependence graded or buffered/thresholded, as the existing SHMT2 loss-of-function data suggest?

Does the MTHFR C677T polymorphism, which redistributes folate flux between the reductive and oxidative branches, produce reciprocal effects on the SAM-dependent (DNA, RNA) versus 10-formyl-THF-dependent (protein) arms of the formyl axis?

Do IDH1/2-mutant cancers, in which α-hydroxyglutarate inhibits both TET and ALKBH1, exhibit combined deficiencies in 5fC-dependent $tRNA_i^{Met}$ transcription and f^5C_{34}-dependent mitochondrial decoding?

Do inherited neurometabolic and mitochondrial diseases perturb one or more formyl layers? Since mitochondrial diseases are among the most common inherited neurometabolic disorders, patient-derived iPSC-differentiated neurons or organoids could be used to monitor formyl changes.

Can interventions that simultaneously increase one-carbon supply and αKG, including defined dietary or metabolic interventions, alleviate symptoms in model systems of mitochondrial disease? A key prediction is that symptomatic improvement, if observed, should be accompanied by measurable changes in one or more formyl layers.

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Could formyl axis measurements become biomarkers for stratifying metabolic disorders? Combined measurements of formylation levels may help distinguish disorders with superficially similar metabolic phenotypes but different underlying regulatory defects.

Is 5fC enrichment at $tRNA_i^{Met}$ gene clusters a developmental phenomenon restricted to zygotic genome activation, or does it occur in other contexts of translational scaling? Relevant settings include T cell activation, stem cell differentiation, tissue regeneration, aging, and metabolic reprogramming in cancer, where changes in OCM, mitochondrial activity, and translational demand are tightly coupled.