Molecules and Cells

Journal Club

Where Does N-Formylmethionine Come from? What for? Where Is It Going?

What is the origin of N-formylmethionine in eukaryotic cells?

Chang-Seok Lee[1,2], Dasom Kim[1,2], and Cheol-Sang Hwang[1,*]

[1]Department of Life Sciences, Pohang University of Science and Technology, Pohang 37673, Korea, [2]These authors contributed equally to this work.
*Correspondence: cshwang@postech.ac.kr
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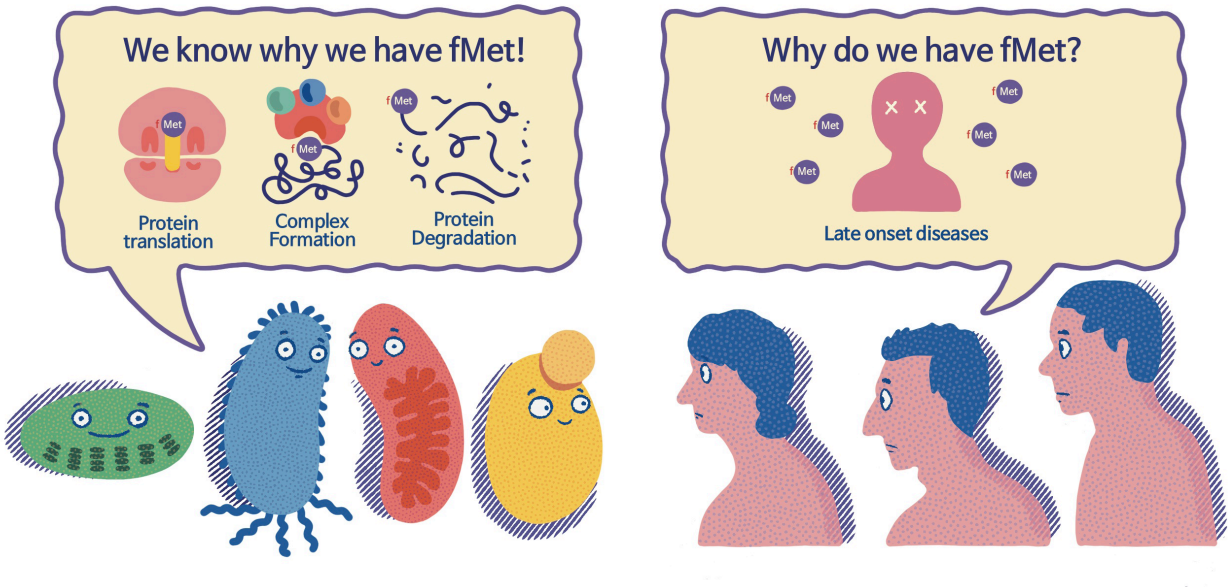

Different roles of N-formylmethionine (fMet) in different species and organelles. fMet was initially found in the translation process of bacteria, chloroplast, and mitochondria. Moreover, fMet promotes protein complex formation in mitochondria and works as a degradation signal in bacteria and yeast. Although the role of fMet in humans remains unresolved, a positive correlation between fMet, integrated stress response, and late-onset diseases has been identified. These unexpected results will guide further functional analysis of fMet in humans.

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One of the fundamental questions of molecular biology was defining the starting signal for translating mRNA into protein. An unexpected observation was the over-representation of methionine (Met) at the N-terminal (Nt) position for the vast majority of bacterial proteins, rather than the *a priori* expected equal distribution of the 20 amino acids at this position (Waller, 1963). Subsequently, Marcker and Sanger (1964) discovered N-terminally formylated fMet-tRNA (formally denoted as fMet-sRNA). They hypothesized that fMet would be a critical factor for translation initiation, with the Nt-formyl moiety blocking fMet incorporation within the internal position of the nascent polypeptide (Marcker and Sanger, 1964). Indeed, fMet-tRNA was confirmed to be crucial for the initiation of protein synthesis in *Escherichia coli* extracts (Adams and Capecchi, 1964). Thereafter, formyltransferase (FMT) was purified and shown to recruit 10-formyl-tetrahydrofolate as a cofactor for the formylation of Met-tRNA (Dickerman et al., 1967). fMet-tRNA was also found in ancient bacteria-derived eukaryotic symbionts, such as mitochondria (Smith and Marcker, 1968). However, no fMet-tRNA, FMT, or fMet-bearing nascent polypeptides were detected in the cytosol of eukaryotic cells, wherein protein synthesis occurred with a Met-tRNA without Nt-formylation (Housman et al., 1970). Consequently, fMet has been widely accepted as a hallmark of bacteria or bacteria-derived organelles, such as mitochondria and chloroplasts.

Interestingly, a recent study pinpointed a substantial link between cellular fMet and the risk of age-associated illness in humans (Cai et al., 2021). Moreover, Cai et al. (2021) provided the first defined molecular evidence bridging mitochondrial DNA (mtDNA) variants and human health, which was achieved by surveying 16,220 individuals and assessing their mtDNA variations and resulting molecular and metabolic differences. These researchers developed a haplogroup lineage tree of mtDNA SNPs (single nucleotide polymorphisms) and identified specific lineages that possess a positive or negative correlation with the fMet level. Specifically, individuals with mt.1811A>G in mitochondrially encoded 12S rRNA and mt.1189C.T in mitochondrially encoded 16S rRNA—a part of the haplogroup U and K (Uk) lineage—showed an increase in the fMet level. In contrast, mt.3992C>T in mitochondrially encoded NADH: ubiquinone oxidoreductase core subunit 1—a part of the mtDNA haplogroup H4 lineage—showed a decrease in the fMet level.

To explore the implications of fMet on human physiology, they generated a transmitochondrial cybrid—an artificial cell fusion between a cell depleted of mtDNA and cytoplast/mtDNA from either the Uk or H haplogroups—and observed a direct effect of mtDNA from the two different haplogroups. Similar to the *in vivo* data, Uk cybrids showed a higher fMet level than that of the H cybrids. Interestingly, upregulation of fMet in the Uk cybrids was independent of the expression level of proteins associated with one-carbon metabolism, degradation by peptide deformylase, or mitochondria protein translation efficiency (Cai et al., 2021).

To further understand the *in vivo* function of fMet, both the Uk and H cybrids were supplemented with exogenous fMet. Unexpectedly, this supplementation caused a significant increase in the intracellular fMet concentration. Furthermore, increasing supplementation with fMet resulted in a variety of cellular responses, such as reduced mitochondrial translation, oxidative phosphorylation, complex I/IV formation, and mitochondrial respiratory capacity. Additionally, fMet supplementation unexpectedly triggered the cytosolic integrated stress response (ISR) while having no effect on mitochondrial unfolded protein response and showed upregulation of polyubiquitylated proteins.

Based on previous reports showing an anticorrelation between haplogroup Uk and various risks of late-onset disorders (Chinnery et al., 2010; Hudson et al., 2013), the researchers were interested in determining the role of fMet in those pathological conditions. Measurement of blood fMet levels from ischemic stroke (IS) cohorts of haplogroup Uk revealed a negative correlation between fMet and the risk of IS in a mtDNA haplogroup-dependent manner. Furthermore, statistical analysis defined a positive correlation between fMet concentration and the risk of various age-associated illness, including heart failure and coronary artery disease. This indicates that fMet is a potential biomarker for various age-related diseases (Cai et al., 2021).

In light of the novel implications of self-generated fMet in the pathophysiology of organisms, a recent study (Cai et al., 2021) revealed the ground-breaking possibility of fMet as a signaling molecule that affects mitochondria and the cytoplasm, thus providing a possible explanation for the conservation of fMet across multiple species. Although the significance of this study should not be overlooked, many questions remain unanswered. First of all, the origin of fMet is still enigmatic. Given the nature of Nt-formylation, plausible sources include fMet-tRNA or fMet-proteins; although, there are no known enzymes that specifically promote fMet cleavage. Nonetheless, our previous work identified that the fMet/N-degron pathway specifically targets fMet-containing proteins for ubiquitin-dependent degradation (Kim et al., 2018). Such formyl-specific proteolysis may regulate fluctuations in endogenous fMet levels. Further studies are warranted to validate the presence of the fMet/N-degron pathway and its relationship to cellular fMet level in mammalian systems.

Moreover, cytosolic fMet-protein translation is a possibility that cannot be ruled out. Due to the limited number of fMet-proteins synthesized in mitochondria (8 in yeast and 13 in humans) (Kummer and Ban, 2021), it is unlikely that all fMets detected are solely derived from mitochondria. Furthermore, mitochondrial fMet-proteins are compartmentalized locally, and there is no evidence that their retrotranslocation into the cytoplasm occurs without mitochondrial rupture (Raoof et al., 2010). Consequently, the source of fMet is most likely cytoplasmic. We proposed that mitochondrially localized FMT shifts its location under cellular stresses, such as nutrient deprivation and activates cytosolic fMet-protein translation (Kim et al., 2018). Therefore, cytoplasmic fMet-protein translation may be involved in fine-tuning the endogenous fMet concentration. In the future, it would be interesting to investigate the association between cytosolic fMet-protein translation, endogenous fMet levels, and the risk of the aforementioned human disorders.

The function of fMet in human disorders remains unresolved. Nonetheless, Cai et al. (2021) have shown a correla-

tion between cellular fMet levels and ISR, indicating that fMet interferes with cytosolic stress responses. The positive correlation of fMet with numerous human diseases can be rationalized since ISR is a signature of human diseases, including ischemia (Zhang et al., 2021). Therefore, fMet may act as an agonist of stress-responsive kinases, which are upstream of ISR; however, further research is warranted to clarify this role of fMet.

In sum, fMet is no longer a hallmark of bacteria or bacteria-derived organelles. The previously unattractive form of fMet, free fMet, has been found to have a unique role in human cells. For years, the physiological role of fMet has been overlooked, and this recent study (Cai et al., 2021) has linked fMet to various human pathologies. Although the suggested underlying molecular mechanism(s) is unclear, addressing the aforementioned questions will shed further light on the importance of fMet in pathophysiology and why evolution opted to conserve fMet across various species.

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AUTHOR CONTRIBUTIONS

C.-S.L., D.K., and C.-S.H. wrote the paper.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ORCID

Chang-Seok Lee https://orcid.org/0000-0001-5249-3466
Dasom Kim https://orcid.org/0000-0001-6389-0950
Cheol-Sang Hwang https://orcid.org/0000-0002-0105-5957

REFERENCES

Adams, J.M. and Capecchi, M.R. (1966). N-formylmethionyl-sRNA as the initiator of protein synthesis. Proc. Natl. Acad. Sci. U. S. A. *55*, 147-155.

Cai, N., Gomez-Duran, A., Yonova-Doing, E., Kundu, K., Burgess, A., Golder, Z., Calabrese, C., Bonder, M., Camacho, M., Lawson, R., et al. (2021). Mitochondrial DNA variants modulate N-formylmethionine, proteostasis and risk of late-onset human diseases. Nat. Med. *27*, 1564-1575.

Chinnery, P.F., Elliott, H.R., Syed, A., Rothwell, P.M., and Oxford Vascular Study (2010). Mitochondrial DNA haplogroups and risk of transient ischaemic attack and ischaemic stroke: a genetic association study. Lancet Neurol. *9*, 498-503.

Dickerman, H.W., Steers, E., Jr., Redfield, B.G., and Weissbach, H. (1967). Methionyl soluble ribonucleic acid transformylase. I. Purification and partial characterization. J. Biol. Chem. *242*, 1522-1525.

Housman, D., Jacobs-Lorena, M., Rajbhandary, U.L., and Lodish, H.F. (1970). Initiation of haemoglobin synthesis by methionyl-tRNA. Nature *227*, 913-918.

Hudson, G., Nalls, M., Evans, J., Breen, D., Winder-Rhodes, S., Morrison, K., Morris, H., Williams-Gray, C., Barker, R., Singleton, A., et al. (2013). Two-stage association study and meta-analysis of mitochondrial DNA variants in Parkinson's disease. Neurology *80*, 2042-2048.

Kim, J., Seok, O., Ju, S., Heo, J., Yeom, J., Kim, D., Yoo, J., Varshavsky, A., Lee, C., and Hwang, C. (2018). Formyl-methionine as an N-degron of a eukaryotic N-end rule pathway. Science *362*, eaat0174.

Kummer, E. and Ban, N. (2021). Mechanisms and regulation of protein synthesis in mitochondria. Nat. Rev. Mol. Cell Biol. *22*, 307-325.

Marcker, K. and Sanger, F. (1964). N-formyl-methionyl-S-RNA. J. Mol. Biol. *8*, 835-840.

Raoof, M., Zhang, Q., Itagaki, K., and Hauser, C. (2010). Mitochondrial peptides are potent immune activators that activate human neutrophils via FPR-1. J. Trauma *68*, 1328-1334.

Smith, A.E. and Marcker, K.A. (1968). N-formylmethionyl transfer RNA in mitochondria from yeast and rat liver. J. Mol. Biol. *38*, 241-243.

Waller, J.P. (1963). The NH2-terminal residues of the proteins from cell-free extracts of E. coli. J. Mol. Biol. *7*, 483-496.

Zhang, G., Wang, X., Rothermel, B., Lavandero, S., and Wang, Z. (2021). The integrated stress response in ischemic diseases. Cell Death Differ. 2021 Nov 6 [Epub]. https://doi.org/10.1038/s41418-021-00889-7