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Genomics update

One-carbon metabolism, folate, zinc and translation

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Summary

The translation process, central to life, is tightly connected to the one-carbon (1-C) metabolism *via* a plethora of macromolecule modifications and specific effectors. Using manual genome annotations and putting together a variety of experimental studies, we explore here the possible reasons of this critical interaction, likely to have originated during the earliest steps of the birth of the first cells. Methionine, S-adenosylmethionine and tetrahydrofolate dominate this interaction. Yet, 1-C metabolism is unlikely to be a simple frozen accident of primaeval conditions. Reactive 1-C species (ROCS) are buffered by the translation machinery in a way tightly associated with the metabolism of iron–sulfur clusters, zinc and potassium availability, possibly coupling carbon metabolism to nitrogen metabolism. In this process, the highly modified position 34 of tRNA molecules plays a critical role. Overall, this metabolic integration may serve both as a protection against the deleterious formation of excess carbon under various growth transitions or environmental unbalanced conditions and as a regulator of zinc homeostasis, while regulating input of prosthetic groups into nascent proteins. This knowledge should be taken into account in metabolic engineering.

Introduction

In all three domains of life, protein synthesis begins with the insert of a methionine residue at the N-terminal end of the polypeptide *in statu nascendi* (Marintchev and Wagner, 2004). In the Bacteria domain and in organelles of Eukarya, this first amino acid is further tagged by a formyl group derived from 10-formyl-tetrahydrofolate (Sinha *et al*., 2014; Shetty *et al*., 2017). In parallel, besides methionine-derived modifications, several tRNA nucleotide modifications are derived from folate-dependent one-carbon (1-C) groups. This implies that protein synthesis is always tied up to the 1-C metabolism, a well-defined subset of intermediary metabolism. This fact, the functional reason of which is not understood, has considerable implications in terms of biotechnology applications, namely for metabolic engineering (Schwechheimer *et al.,* 2018). This metabolic cornerstone is also reflected in the conservation of critical functions that are encoded in most genomes, including the smallest ones (Danchin and Fang, 2016). Yet, the underlying reasons for this fairly enigmatic metabolic coupling have not been explored in genome-wide studies.

A variety of experiments delineated, in various genetic backgrounds and environmental conditions, the frontiers of methionine tagging by a formyl group in Bacteria and in eukaryotic organelles, but their scope was limited. It was observed early on that Firmicutes could dispense of formylating their initiator methionyl-tRNA provided the growth medium was supplemented with all the metabolites directly associated with the 1-C metabolism [serine/glycine, purines, thymine, pantothenate (Samuel *et al*., 1970)]. While this process was not in force in gammaproteobacteria, in particular in *Escherichia coli*, it was observed that *thyA* mutants – unable to synthesize thymine *de novo*, as well as a variety of other mutants, allowed growth of these bacteria in conditions similar to those explored for Firmicutes (Danchin, 1973; Harvey, 1973). Supporting an involvement of folic acid metabolism, the main molecular event allowing this competence in Firmicutes was that replacement of the thymine

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residue of the TΨCG motif in the T-loop tRNAs by its uracil precursor allowed formyl-less translation initiation when the tetrahydrofolate-dependent metabolism was inactivated. It was further demonstrated that, in contrast to other bacteria where this residue is methylated using S-adenosylmethionine (AdoMet) by enzymes of the TrmA family (Ranaei-Siadat *et al*., 2013), an enzyme of a different descent, using 5,10-methylene-tetrahydrofolate (CH_2-N^5,N^{10}-H_4F) and a reduced flavin, was performing this unique methylation step (Delk and Rabinowitz, 1974, 1975). This identified yet another function where the 1-C H_4F-dependent metabolism was associated with translation. Here, we explore further in depth this relationship and review the genome data that consistently link together folates, the 1-C metabolism and translation. Having uncovered that zinc homeostasis is a key step in these processes, we propose at the end of this article a rationale for this perhaps unexpected coupling, apparently coordinated by the modification of anticodon base 34 of tRNAs.

Methionine as the main product of folate metabolism

To try and understand the coupling between translation and metabolic pathways, let us review the first steps of the emergence of living cells. Prebiotic chemistry was likely surface-based and relying on amino acid syntheses (Wächtershäuser, 1988). We still witness this origin in that extant biosynthesis of major building blocks of the cell, such as purines or pyrimidines, requires steps that involve amino acids. This metabolic feature is seldom exploited in scenarios based on standard inorganic chemistry. Furthermore, prebiotic metabolites are electrically charged metabolites, prone to stick on surfaces. This is particularly relevant for nucleotides (purines and pyrimidines linked to a ribose and a phosphate) and coenzymes, and contrasts with the current compounds of laboratory-developed chemical reactions, which would be immediately diluted out in their aqueous environment. When comprising carboxylate or phosphate moieties, charged metabolites can be locally concentrated (Wächtershäuser, 1988). In addition, while carbon chemistry involving a small number of atoms is straightforward and indeed widespread in the universe (Ehrenfreund *et al*., 2011), a major question asked to any convincing scenario of the origin of the first cells is that of the origin of compounds carrying multiple nitrogen atoms. In this respect, nitrogen fixation is a critical process that needs to be properly matched with the carbon supply.

Among the many possible scenarios of the origins of life, we retain those where carbon skeleton molecules and sulfur played a major role (Blochl *et al*., 1992), being, for example, associated with a reverse Krebs cycle catalysed by iron–sulfur clusters (Camprubi *et al*., 2017). These first metabolic pathways produced, in addition to omnipresent formate (1-carbon) and glycine (2-carbon), 3-carbon, 4-carbon and 5-carbon metabolites that further associated with a nitrogen-fixing process. An interesting variant scenario makes use of iron–sulfur-rich clay environments containing phosphates, where thioester-based metabolism is the rule (Hartman and Smith, 2019). In this family of scenarios, a thioester swinging arm, such as 4-phosphopantetheine, would be involved in the polymerization of relevant metabolites, such as those generated today in non-ribosomal peptide synthesis, polyketide synthesis or fatty acid synthesis (Lipmann, 1971), resulting in the synthesis of coenzymes, pterins in particular (Danchin, 2017a). While this latter view may appear far-fetched, extant metabolism has identified in at least one case a bacterium displaying an explicit link between non-ribosomal peptide synthesis and pteridine synthesis (Park *et al*., 2017). Nitrogen fixation had also to store nitrogen-rich compounds, possibly as guanidinium skeleton-containing metabolites. Among those, folates, flavins, pteridines and molybdopterins may have been important stores and intermediates in the synthesis of further essential building blocks, notably the direct synthesis of guanylate (ribose included), from folic acid derivatives and formate, one of the most frequent carbon compounds in the universe (Danchin, 1989). This metabolic step would carry on the whole of 1-C group-mediated metabolism. Note that this scenario is also consistent with the fact that, in extant metabolism, the hydroxymethyltransferase that builds up ketopantoate – a core element of the phosphopantetheine arm – is a tetrahydrofolate-dependent enzyme (Chaudhuri *et al*., 2003). To be sure, it provides a good example of an autocatalytic cycle of the graded autocatalysis replication domain (GARD) type (Lancet *et al*., 2018), another important likely step in prebiotic chemistry.

Yet, another feature of folic acid coenzymes further highlights the role of non-ribosomal synthesis of (iso) peptides. It is illustrated in the fact that intracellular folates contain a polyglutamate isopeptide, usually consisting of 5–8 glutamate residues that are polymerized through unusual γ-linked peptide bonds. The polyglutamate moiety is critical for optimal activity of folate-dependent enzymes. This modification allows sequestering of folate within the cell, and, as witnessed in thioester-dependent syntheses using 4-phosphopantetheine, it may serve as a swinging arm that permits metabolic channelling of the cofactor between successive folate-dependent enzymes or catalytic centres (Schirch and Strong, 1989). Unravelling the possible scenarios coupling all these pathways together in an autocatalytic cycle (Kahana and Lancet, 2019) is out of the scope of the present work. Let us simply assume that, at some point, these processes resulted in the folate-dependent

synthesis of methionine following one of the many pathways involving the protection/ deprotection steps that are still distributed among extant organisms that make methionine (Ferla and Patrick, 2014; Bastard *et al*., 2017). In these scenarios, nucleotide polyphosphates were simultaneously available (Deamer, 2017), and this permitted the synthesis of AdoMet, a critical ubiquitous metabolite present in all cells and the major donor of one-carbon methyl groups – because of its intrinsic reactivity as a sulfonium species.

This general picture implies that the 1-C metabolism was fully functional in prebiotic times, based on a number of 1-C-carrying folate coenzyme derivatives. The sequel of this metabolism is still open to investigation. Most extant 1C-folate compounds belong to a series involving the highly reduced form, tetrahydrofolate [H_4F (Fig. 1)]. Among those, only two compounds are fairly stable (Gregory, 2012; Zheng and Cantley, 2019), namely 5-methyl-tetrahydrofolate (CH_3-N^5-H_4F) and 5-formyl-tetrahydrofolate (CHO-N^5-H_4F). The latter seems to be a dead-end store product that does not appear to be directly involved in a significant number of biochemical reactions, except for its isomerization into the metabolically reactive form, 10-formyl-tetrahydrofolate (CHO-N^{10}-H_4F). By contrast, CH_3-N^5-H_4F is widely used as a precursor of methionine, using homocysteine as substrate either directly or indirectly via a coenzyme B12-mediated reaction. As a consequence, methionine and AdoMet are the main output of folic acid-mediated metabolism, followed by purines. Besides these metabolites, thymine is another critical 1-C metabolite, but its role is confined to DNA synthesis, or sometimes to the synthesis of a fraction of carbohydrate-related pathways (Hosono *et al*., 1975). This makes that the amount of thymine available for the cell's metabolism is submitted to tight control. However, its synthesis is often coupled to all other 1-C metabolites via a pathway that introduces a huge leverage effect. To be sure, in most organisms, thymine *de novo* synthesis (using ThyA-like enzymes) requires tetrahydrofolate as a substrate, not as a recycled coenzyme. This makes that, despite its relatively low metabolic burden, DNA synthesis is usually strongly coupled to all processes involving 1-C metabolism. We further note here that, overall, in parallel with pathways that produced CO_2 as a final waste product, other one-carbon compounds – methane, methanol, formaldehyde or formate – are produced in excess and disposed of under many conditions, a situation that may account for the involvement of H_4F compounds in buffering 1-C excess steps in the cell's metabolism. As a case in point, CHO-N^{10}-H_4F is oxidized to CO_2 in mitochondria using a NADP-dependent aldehyde dehydrogenase [*e.g.* ALDH1L2 in human cells (Zheng and Cantley, 2019)]. This is particularly important for the control of formaldehyde, which, in contrast to CO_2, is a toxic metabolite because of its propensity to react with amino groups.

Methionine as the first amino acid in translation

Methionine is the first amino acid residue of all polypeptides translated by the ribosome nanomachine. This feature is common to all three domains of life and therefore expected to witness the metabolic conditions that prevailed when the process of translation emerged in the first cells. Yet, nothing in the physical chemistry of this amino acid appears to justify this remarkable role in any straightforward way. In particular, the fact that methionine comprises a sulfur atom does not appear to be relevant in any way to the translation initiation step. However, among the possible features that could be retained by this process is the fact that methionine's side-chain is highly flexible – it can be seen as a lubricating oil drop – hence prone to adapt to a variety of mechano-chemical constraints, especially in a hydrophobic environment (Fischer *et al*., 2013; Gorbitz *et al*., 2016). That this feature is relevant is witnessed by the fact that *n*-norleucine, an amino acid isosteric to methionine, can replace the latter, not only within the polypeptide chain (Cohen and Munier, 1956; Anfinsen and Corley, 1969), but also as an amino acid used in initiation of protein synthesis (Brown, 1973). This can be interpreted as implying, at least for this very specific function, that methionine was retained for translation initiation as a frozen accident of prebiotic metabolism because it was readily available as a versatile plastic N-terminal residue. This also substantiates the view that methionine – and therefore the 1-C metabolism – had been present during the unfolding of the prebiotic chemistry that led to translation.

Yet, methionine biosynthesis is fairly costly in terms of metabolic demands. It requires a redox-neutral or reducing environment (which is consistent with prebiotic life) that depends on the tetrahydrofolate [or in some cases, tetrahydromethanopterin (Ragsdale, 2008; Deobald *et al*., 2020)] cycle and, sometimes, on the presence of coenzyme B12 derivatives. This latter requirement makes the process of homocysteine methylation considerably more efficient than with the sole use of CH_3-N^5-H_4F, but at a very high genetic and energy cost (Fig. 1). Retaining the *N*-terminal methionine solely at the beginning of all proteins would have been a major burden in terms of biomass if methionine were not also an integral component of polypeptides, this time selected for a variety of functions, in particular for its ability to bind copper ions (Meir *et al*., 2019). Yet, the constant requirement for starting polypeptides with a methionine residue triggered the ubiquitous presence of an essential methionine aminopeptidase activity, which evolved to recycle

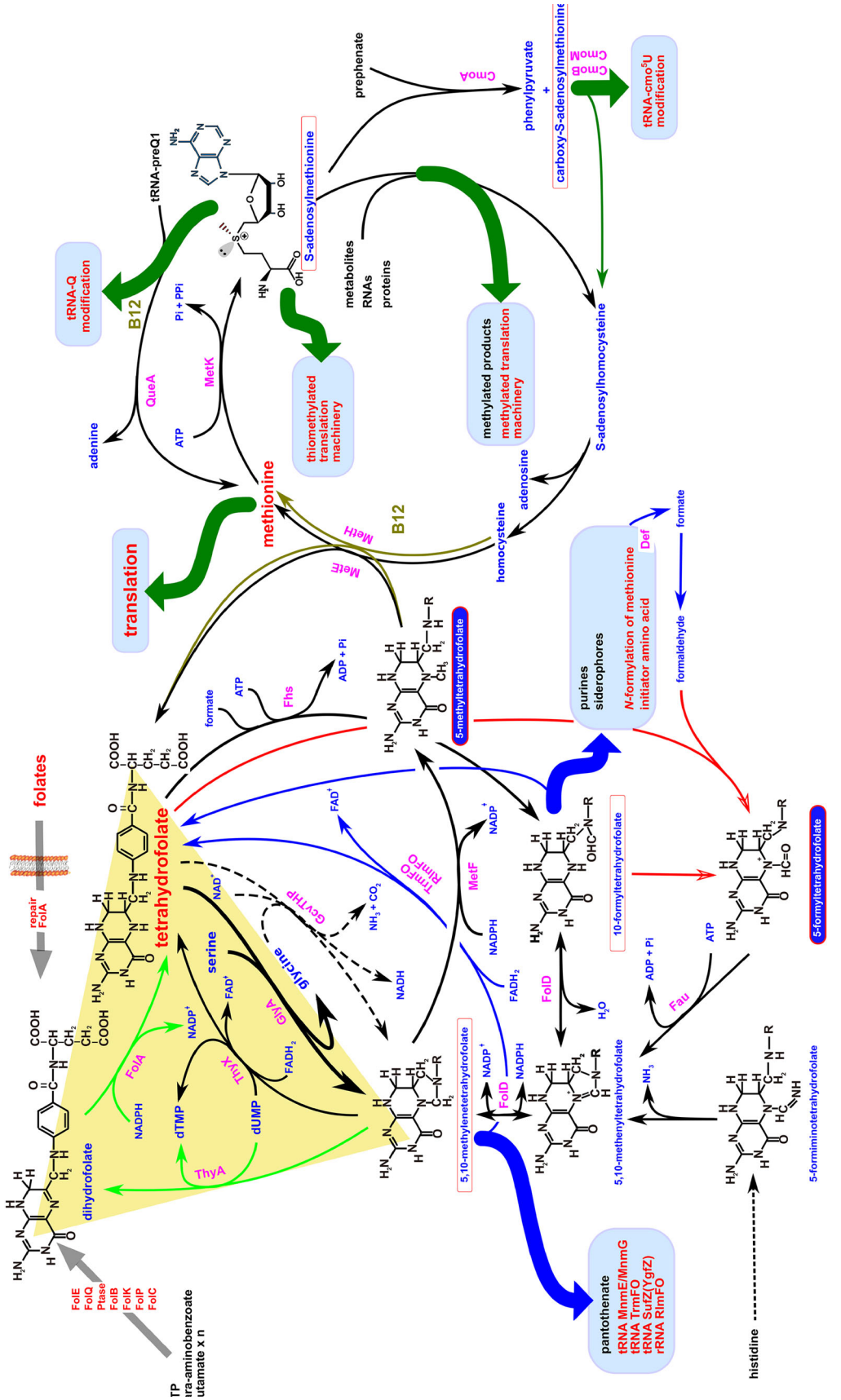

Fig. 1. General outline of one-carbon metabolism. The bulk of one-carbon supply comes from the hydroxymethyl group of serine in the form of CH_2-N^5,N^{10}-H_4F (thick black arrow). This initiates a cycle that is used to donate 1-C groups to a variety of substrates, with methionine as the major final metabolite. In turn, methionine is the precursor of AdoMet, which is used in a large number of reactions, dominated by methylations. Thick blue arrows summarize the main direct outputs 1-C groups with macromolecule targets indicated in red. Red arrows display spontaneous reactions, while light green arrows show the cycle recycling H_4F via H_2F. The yellow triangle shows the set of reactions where H_4F is used as a substrate, not simply as a coenzyme. Thick dark green arrows summarize the main output from the methionine/ AdoMet cycle. See text for details.

methionine in all extant organisms. This enzyme often acts in a co-translational way, thus allowing the maintenance of a significant methionine pool, immediately available for further metabolism (You *et al*., 2005; Lechner *et al*., 2011; Danchin and Fang, 2016). Its key role has been exploited by fungi via the synthesis of fumagillin, an antimicrobial compound that targets the methionine aminopeptidase type 2 (MetAP2) enzyme (Guruceaga *et al*., 2019).

Methionine is also the precursor of AdoMet, yet another essential 1-C metabolite that plays a wide variety of critical roles [to our knowledge not yet reviewed in any extensive survey, see Clarke, 1993; Chiang *et al*., 1996; Grogan and Cronan, 1997; Cohen, 1998; Marsh *et al*., 2004; Roje, 2006; Marsh *et al*., 2010; Struck *et al*., 2012; Zhang *et al*., 2012; Lanz and Booker, 2015; Yokoyama and Lilla, 2018 for an incomplete survey of relevant activities, dominated by methylation processes]. As expected, the *metK* gene is essential unless AdoMet is imported into cells where MetK has been inactivated (Tucker *et al*., 2003). As a consequence, methionine sparing required also the emergence of an important cycle involving the AdoMet-related activities producing the methylthioadenosine metabolite. This resulted in omnipresent methionine salvage pathways (Sekowska *et al*., 2019). In point of fact, AdoMet synthesis and turnover makes use of approximately three to four times the free methionine pool in steady-state unsupplemented conditions (Thomas *et al*., 1988; Shlomi *et al*., 2014). All this highlights the key role of this 1-C metabolite, which is involved in a large number of reactions that have an ancient origin (Sousa *et al*., 2016). In particular, the translation machinery asks for ubiquitous methylation steps involving ribosomal RNAs, transfer RNAs and ribosomal proteins, as we document further below.

The formylation of methionine-loaded initiator tRNA, its role and its fate

Following the discovery that methionyl-tRNA ('soluble' RNA, sRNA, at the time) was formylated in a bacterial cell-free system, Clark and Marcker initiated a study of the role of fMet-tRNA and discovered that a distinct species of $tRNA_{Met}$ that could be loaded with methionine was involved in the initiation of protein synthesis ($tRNA^{F}_{Met}$), while a second species incorporated the amino acid within the polypeptide chain [$tRNA^{M}_{Met}$ (Clark and Marcker, 1966)].

Formylation of initiator methionine

A large number of studies followed. They established that formylation was omnipresent in Bacteria but absent from Archaea and from Eukarya [except in their organelle's translation machinery, mitochondria and chloroplasts, e.g. in *Euglena gracilis* (Schwartz *et al*., 1967)]. The tetrahydrofolate-dependent pathways leading to 1-C groups were deciphered at the same time (Fig. 1). Their anabolic role was emphasized by the fact that the corresponding redox reactions depended on NADP, not NAD (Albrecht *et al*., 1968). Early experiments made also apparent that in organisms such as *Streptococcus faecium*, translation initiation in the absence of folate did not require this formylation step (Pine *et al*., 1969). Casting some doubt about the significance of the modification, this observation triggered a number of studies that explored the conditions under which formylation was apparently essential. Firmicutes differed from *E. coli* in that, while formylation was indispensable in the latter, it could be dispensed of in Streptococci or Bacillus species. As discussed in Introduction of this article, a detailed analysis pointed out that the T-stem and loop of $tRNA^{F}_{Met}$ was crucial in this process at least in Firmicutes. Further studies showed that in *E. coli*, the same low methylation level of the ribothymidine of the T-loop resulted again in the ability for cells to grow without formylation in mutants without folate [in fact, depleted of para-aminobenzoic acid (Baumstark *et al*., 1977)]. Again, this pointed out the existence of a tight link between 1-C metabolism and initiation of translation.

This unique feature is prevalent in Bacteria and reflected in the usual presence a formyl-methionine transferase and one or several deformylases, witnessed, for example, in bacteria of distant clades such as *E. coli* (diderm) and *Bacillus subtilis* (monoderm). There, these enzymes are encoded within the *def-fmt-rsmB* operon, comprising another translation-related gene, *rsmB*, that encodes a ribosomal RNA methylase (discussed below). What could then be the role of this formyl group? Besides further tying up 1-C metabolism to the general process of translation, this group could have a regulatory role in the initiation step, conceivably identified via the study of mutants that grow without methionine formylation. Genetic studies showed that, besides direct involvement in translation, the 1-C metabolism was coupling translation with replication by way of thymine synthesis, which follows an unusual course. In contrast to all other roles of tetrahydrofolate compounds in metabolism, synthesis of thymine in all organisms that do not use ThyX enzymes uses CH_2-N^5,N^{10}-H_4F as a *substrate*, not as a coenzyme (Koehn *et al*., 2009). To be sure, the reaction does not recycle the factor – as expected for a coenzyme – but yields dihydrofolate that must subsequently use dihydrofolate reductase to be reduced to tetrahydrofolate again (Fig. 1). As suggested previously, this confers a considerable leverage effect of thymine synthesis on all metabolic pathways involving 1-C derivatives.

Further experiments also revealed an unexpected link with transcription, witnessed by growth of various RNA polymerase mutants in the absence of formylation (Danchin, 1973). tRNA appears indeed to bind to RNA polymerase, but with no direct role of formylated methionine, so that the link between transcription and modification of the N-terminal methionine of polypeptides remains to be understood [(Spassky *et al*., 1979); see, however, Nomura *et al.* (1986), no further recent studies on this topic yet].

Transcription–translation coupling

In translation, early experiments suggested that the formyl tag of initiator methionine was used as an allosteric effector that allowed 70S ribosomes to be reset to an initiator state, without prior dissociation into a 30S + 50S pair (Petersen et al., 1976a, b). This was expected to modulate the relative expression of genes in polycistronic operons, thus coupling the 1-C metabolism with polarity of gene expression, a subtle way to adapt gene expression dosage to metabolism (Petersen *et al*., 1978). This role was not further explored for a long time, until, four decades later, Yamamoto and co-workers established that 70S-mediated initiation was indeed a frequent mode of translation initiation in bacteria (Yamamoto *et al*., 2016). This recent work was further substantiated by showing that the termination phase of translation of a cistron was not obligatorily followed by a translation release factor-dependent recycling of the ribosomes (Qin *et al*., 2016), allowing 70S ribosomes to proceed undissociated for translation initiation, accepting formylated methionine-loaded $tRNA_{Met}^{F}$ directly at the peptidyl site. As such, a role of 1-C metabolism would be, in the Bacteria domain, to modulate the relative expression of proteins encoded in polycistronic operons. It will therefore be of interest to compare the structure of operons between organisms that have maintained this coupling and those that did not. To our knowledge, this constraint has not yet been explored in construction of metabolic operons for biotechnology purposes.

Polypeptide N*-end degradation*

The outcome of the formylation process controlling translation initiation is that polypeptides begin with a formylmethionine start. Obviously, this must interfere with the *N*-terminal recycling of methionine and with regulation involving *N*-terminus-mediated protein degradation [*N*-end degrons (Humbard *et al*., 2013; Piatkov *et al*., 2015)]. That this is an important process in Bacteria is reflected by the emergence in Streptomycetes of an antibiotic, actinonin, that inhibits peptide deformylation (Chen *et al*., 2000). This role is true not only in Bacteria but also in eukaryotic organelles, as witnessed in chloroplasts, for example (Serero *et al*., 2001). However, in mitochondria it operates via a complex quality control of mitochondrial proteins that does not appear to involve deformylase but, rather, a mimic of a formylated factor involved in mitoprotein quality control (Richter *et al*., 2015; Battersby *et al*., 2019). Proteins affected by formylation-sensitive degradation have been identified, for example in *B. subtilis*. A variety of functions in Fmt-less bacteria are defective for several post-exponential phase adaptive programmes including antibiotic resistance, biofilm formation, swarming and swimming motility, or sporulation. In addition, a survey of well-characterized stress responses showed an increased sensitivity to metal ion excess and oxidative stress (Cai *et al*., 2017). Formylation is also essential for normal growth in Mycoplasma species, but not for viability (Vanunu *et al*., 2017). All these observations point out a role that connects together a subset of biological functions that are very important, yet generally not strictly essential.

As previously noted, the organelles' protein synthesis involves *N*-formylation. This modification has only a minor functional role in stable steady-state conditions (Franco *et al*., 2019), but it is required for some critical functions, possibly related to environmental transitions and involving the regulation of protein turnover (Battersby *et al*., 2019). Among important processes in human cells, the *N*-terminal formyl-methionine residue of factor COX 1 is required for the correct assembly of cytochrome c oxidase (Hinttala *et al*., 2015). The role of formylation has been explored by inactivating genes that control synthesis of glycine in mitochondria, notably glycine *C*-acetyltransferase – involved in catabolism of threonine and producing glycine – and serine hydroxymethyltransferase. Inactivation of the latter led to generation of embryonic lethal animals, while the former, despite a negative outcome, did not. Both allow synthesis of glycine, but only the latter – which produces CH_2-N^5,N^{10}-H_4F – is used in controlling translation initiation. Again, the importance of formylation has been disputed, but mutants lacking formyltransferase displayed a variety of vital phenotypes. This has been interpreted as substantiating the critical role of this modification in organelles (Tucker *et al*., 2011; Tani *et al*., 2018), which has been considerably boosted by exploration of the role of actinonin, as discussed previously. It must be emphasized that laboratory conditions are meant to be very stable and certainly do not mimic life in a world where organisms continuously face multiple transitions. Many functions must be linked together in order to cope with transitions and would thus be difficult to visualize in standard laboratory experiments, which in this context are in fact closer to artefacts than to proper living conditions.

Remarkably, the organelle's sensitivity of protein degradation to *N*-blocking has been extended outside the organelles of Eukarya by an unexpected contribution in the cytosol. Indeed, the process mediating the *N*-blocking of the terminus of a number of proteins in organelles also seems to be involved in modification of proteins in the cytosol, somehow using mitochondrial formyltransferase [Fmt1 in *Saccharomyces cerevisiae* (Kim *et al*., 2018)]. Recent experiments showed that these *N*-terminus-modified proteins were massively upregulated in stationary phase or upon starvation for specific amino acids and were crucial for the adaptation to specific stresses. The stress-activated kinase Gcn2 was required for the upregulation of *N*-formylated proteins by modulating the activity of Fmt1 and its retention in the cytosol (Kim, 2019). It will be of major interest to understand whether this process extends to other Eukarya, plants and animals in particular. Pre-translational formylation using fMet-based degradation signals (fMet/*N*-degrons) is likely to be important as well for other processes, such as protein folding during translation. One likely function of fMet/*N*-degrons is the control of protein quality that may develop during polypeptide synthesis well before proteins are released in the cytosol.

Another *N*-blocking process, posterior to translation rather than co-translational, involves C-2 metabolism. *N*-acetylation has been identified in all three domains of life. It is involved in regulating the stress response (Linster and Wirtz, 2018). This process alters *N*-end-triggered protein turnover and even sometimes triggers protein degradation (Oh *et al*., 2017; Nguyen *et al*., 2018; Eldeeb *et al*., 2019). In bacteria, the rate of polypeptide chain elongation is an order of magnitude faster than in eukaryotes. The faster emergence of nascent proteins from bacterial ribosomes is possibly one mechanistic and evolutionary reason for the pre-translational set-up of bacterial fMet/*N*-degrons, in contrast to the co-translational set-up of analogous AcMet/*N*-degrons in eukaryotes (Piatkov *et al*., 2015). However, the newly discovered possibility of *N*-formylation in the cytosol of eukaryotes further opens up the question of the role of this modification. In bacteria, there is also a dialog between *N*-formylation and *N*-acetylation of polypeptides. In these organisms, a large variety of so-called toxin–antitoxin systems regulate gene expression as a consequence of environmental cues. The vast majority of these toxins target protein synthesis. They use a variety of molecular mechanisms and inhibit nearly every step of the translation process. Among those, *E. coli* toxin AtaT is endowed of acetyltransferase activity. This toxin enzyme acetylates specifically the methionine moiety loaded on initiator $tRNA^{F}_{Met,}$ replacing the expected formyl group. This modification drastically impairs recognition by initiation factor 2 (IF2), inhibiting the initiation step of translation (Van Melderen *et al*., 2018), further substantiating that a main role of 1-C metabolism is specifically to regulate initiation of translation.

Other features related to N*-formylation of the translation initiation start*

As a further remarkable feature of *N*-formylation, we notice that pre-translational formylation of peptides introduced in the translation machinery an amino acid with a secondary amine group [formerly named an 'imino' group (Unger and DeMoss, 1966)], a notable exception in the proteogenic amino acids, proline aside. This has considerable consequences in the chemical development of polypeptide synthesis, likely to control the overall speed of the process. To be sure, introducing proline residues in the polypeptide chain slows down translation so much that it becomes difficult or even impossible for runs of proline residues. In the early times of the origin of translation, evolution has selected a specific factor to alleviate this limitation, translation elongation factor EIF5A/EF-P (Woolstenhulme *et al*., 2015; Tollerson *et al*., 2018). Strikingly, this factor is also important for synthesis of a variety of initiator peptides in Bacteria (Aoki *et al*., 1997; Katoh *et al*., 2016), implying that the formylation step is a latecomer that has emerged once this essential compensatory process had evolved so as to accommodate proline in polypeptides.

Finally, polypeptides are processed after they are completed, or even during their synthesis, with a key role of peptide deformylase. This enzyme's activity results in a steady-state flux of reactive 1-C by-products, a fairly puzzling feature knowing that formaldehyde is a toxic metabolite. This is another hint that formylation of initiator methionine is a latecomer in translation-related metabolic pathways. Detoxification of this metabolite is well understood in Methylobacteria (Chistoserdova *et al*., 2007). It is still open to exploration in most bacterial clades, possibly linked to spontaneous reaction of formaldehyde with H_4F – noting, however, that most so-called 'spontaneous' reactions are in fact catalysed by relevant enzyme [see, e.g., hydrolysis of 6-phosphogluconolactone (Miclet *et al*., 2001)]. Deformylation is likely maintained at a sufficiently slow rate, possibly exporting formylated peptides to avoid much of the toxicity of formaldehyde, which is readily produced from formate in the reducing environment of the cytosol. In turn, these peptides act as chemotactic compounds for neutrophils (Hughes *et al*., 1987; Murphy *et al*., 1992; Kretschmer *et al*., 2012; Kurgan *et al*., 2017). They possibly have much wider signalling roles, allowing biotechnological manipulation of quorum sensing in mixed Eukarya/Bacteria populations (Sedlmayer *et al*., 2018).

Yet, a further role of *N*-formylated peptides has evolved in a specific family of microcins that coordinate the development of different bacterial species in microbiota. Microcin C exhibits an obligate requirement for a *N*-terminal formyl group. It is a ribosomally synthesized and post-translationally modified peptide produced by cells harbouring a plasmid with the *mcc* gene cluster. Microcin C is a not hydrolysable heptapeptide-N-P-adenylate that inhibits the growth of sensitive cells. It is transported inside *E. coli* or closely related bacteria by the Hdp(Yej)ABEF transporter, which recognizes the toxic peptide. Subsequently, degraded by aminopeptidases, it generates a toxic, non-hydrolysable aspartamide adenylate that inhibits aspartyl-tRNA synthetase. Remarkably, the adenylation that is essential for the toxin activity is entirely dependent on the formylation of the N-terminal methionine of the heptapeptide (Dong *et al.*, 2019).

AdoMet-dependent methylations of the translation machinery

The ribosome, the nanomachine in charge of polypeptide synthesis, must both be properly shaped and assembled, and be maintained and protected against accidents, as its synthesis requires a considerable amount of building blocks and energy. Furthermore, RNAs are fairly unstable molecules that are highly sensitive to degradation, which implies that relevant folded structures have evolved to be immune to the action of endocytoplasmic RNases. They often need to be further stabilized chemically. Besides agents that use energy to discriminate among the many RNA-folded structure and retain only those that are properly shaped (Tamaru *et al.*, 2018; Boel *et al.*, 2019), ribosomal RNAs are modified, in general via the action of AdoMet-dependent RNA methylases (Sergiev *et al.*, 2018). A few ribosomal proteins (many more in Eukarya than in Bacteria or Archaea) are also modified by such methylases, but these modifications appear to have less essential functions.

The ribosome

Ribosomal RNAs. During the course of RNA folding and assembly of the ribosome, rRNA molecules are methylated to various degrees. We focus here on Bacteria as a telling illustration of the importance of the process. Many of the corresponding bacterial methylases are encoded in the list of genes that make a minimal genome (Table S1). They are well conserved in *Buchnera* sp. (diderms) or Mycoplasmas (monoderms). Most methylations occur on nucleotide bases [*e.g.* in *E. coli* (Sergeeva *et al.*, 2015)], but a few important ones are ribose 2'OH methylations (Monaco *et al.*, 2018; Krogh and Nielsen, 2019). Among these modification enzymes, we retained RsmG, formerly named GidB ('glucose-inhibited division protein B') because it had a phenotype similar to that of GidA (MnmE), a tRNA-modifying regulator discussed below (Shippy and Fadl, 2015), demonstrating a significant interplay between the various modifications of RNA molecules and carbon metabolism. These modifications, widely conserved in evolution (Sergiev *et al.*, 2018), play a role in maturation and function of ribosomal RNA (Nachtergaele and He, 2017; Taoka *et al.*, 2018). They are also likely to protect these essential molecules against degradation, making their metabolic origin – 1-C metabolism – directly tied up to the very process of translation. Finally, there is a significant impact of methylation on ribosome recycling and on fidelity of translation (Seshadri *et al.*, 2009).

Ribosomal proteins. Ribosomal proteins may also be modified by methylation. In *E. coli*, six relevant ribosomal proteins have been identified (Nesterchuk *et al.*, 2011): L3, modified at position 5 of glutamine 150 by methylase PrmB (Lhoest and Colson, 1981), L7/L12, modified at residue lysine 81 by a yet unidentified methylase and L11, modified by the conserved lysine methyltransferase PrmA that trimethylates the *N*-terminal alpha-amino group and the ε-amino groups of Lys3 and Lys39 (Nesterchuk *et al.*, 2011). These modifications appear to be dispensable but may result in a cold-sensitive phenotype in some conditions. However, they seem to be most important in eukaryotic organelles (Mazzoleni *et al.*, 2015). Ribosomal protein methylation is considerably more extensive in Eukarya, with a variety of phenotypes that are not discussed further here.

In addition, a rare methylthiolation is observed in a variety of bacteria. RimO, a methylthiotransferase belonging to the iron–sulfur binding radical AdoMet(SAM) superfamily (Anton *et al.*, 2010; Forouhar *et al.*, 2013), modifies ribosomal protein S12 at aspartate 89 (D88 in the mature protein) in various diderm organisms (Landgraf and Booker, 2016; Molle *et al.*, 2016). When alone, RimO has a reversible action. However, in concert with ATP-dependent factor RimOB (YcaO), the reaction becomes irreversible (Sikandar *et al.*, 2019), witnessing yet another example of a protein that acts as a Maxwell's demon [see Sherrington (1940) p. 78 for an 'animist' attempt to use the concept as a physicochemical metaphor of the cell's life] needed to discriminate the relevant peptide against similar ones, while asking for compulsory energy dissipation to be reset to their original state (Boel *et al.*, 2019).

Translation factors

Elongation factor Tu is monomethylated in *E. coli* during exponential growth at the ε-amino group of lysine 56 residue, then further methylated on this same group

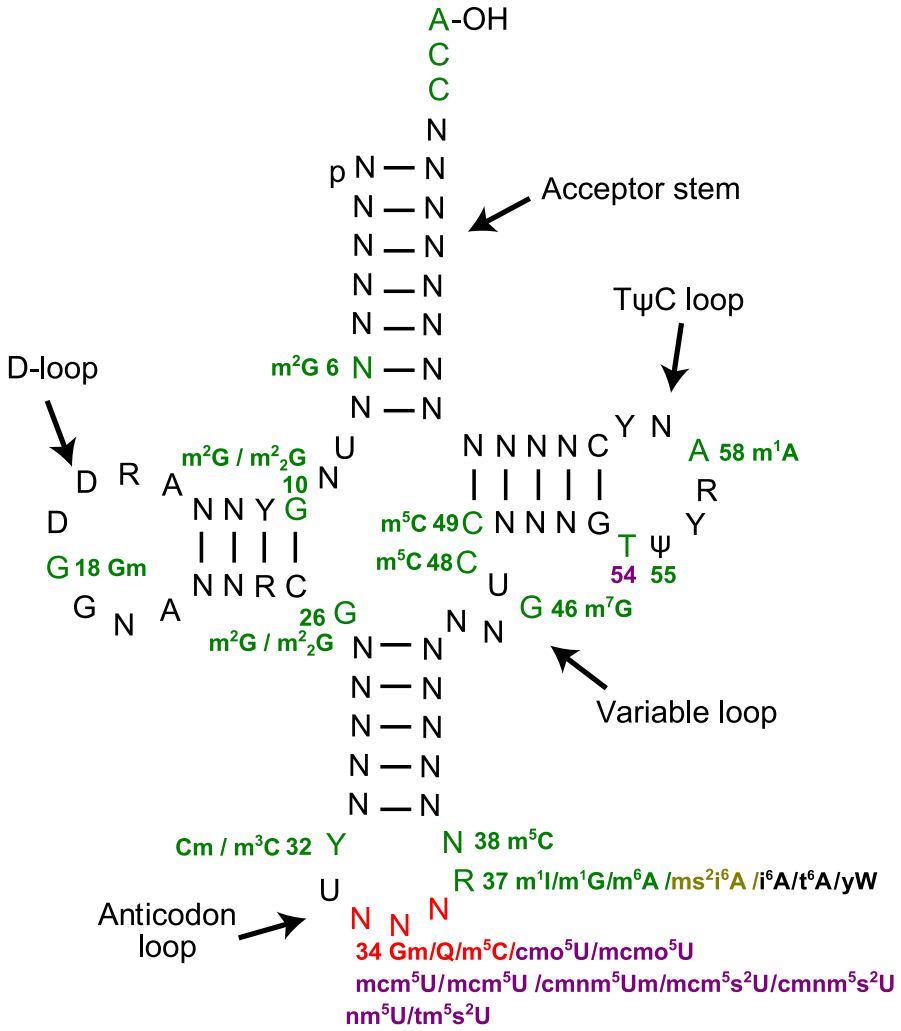

Fig. 2. One-carbon-dependent modifications of tRNA molecules. The anticodon is in red, while the major methylation sites are in green. Modifications that are not derived from 1-C metabolism are in black. U34 modifications are in purple, while thiomethylation of A37 is in brown.

upon entry into stationary phase. Lys56 is located in the GTPase switch-1 region (residues 49-62), a strongly conserved site involved in interactions with the nucleotide and the 5' end of tRNA. Methylation was found to attenuate GTP hydrolysis and may thus enhance translational accuracy by slowing down the process (Kraal *et al.*, 1999). Another methylation of the factor is observed in *Pseudomonas aeruginosa* as a Lysine 5 trimethylation by specific methylase EftM at ambient temperature making this modification important for growth at low temperature (Owings *et al.*, 2016).

The most important 1-C-dependent modification of a translation factor is the methylation of the N^5 position of the glutamine residue belonging to the universally conserved Gly-Gly-Gln (GGQ) tripeptide present in the release factors or release factor-like surveillance proteins required to catalyse the release of peptides *in statu nascendi* from the ribosome (Frolova *et al.*, 1994; Nakahigashi *et al.*, 2002). A remarkable feature of this modification is that it is even conserved in proteins of the three domains of life obviously resulting from convergent evolution. This indicates that the methylation step has remained critical for the translation process throughout evolution (Zeng and Jin, 2018). Besides increasing the overall rate of termination, the effect of this modification seems to depend significantly on the identity of the amino acid residue driving termination, being exceptionally slow on proline and glycine residues, while being accelerated by approximately two orders of magnitude when the GGQ motif is methylated (Pierson *et al.*, 2016).

tRNA modifications

Transfer RNAs (tRNAs) are extensively modified via processes involving the 1-C metabolism, with AdoMet-dependent methylation reactions dominating over a variety of other modifications, thiolations in particular, and some

Table 1. Expression of genes of *E. coli* involved in 1-C metabolism and translation during carbon limitation.

Gene	Strand	Exp_{GL1} (rpkm)[a]	Exp_{GL4} (rpkm)[b]	Exp_{GL1}/ Exp_{GL4}	*P*-value[c]
def	+	374.33	453.93	0.82	0.1185
metH	+	230.18	269.29	0.85	0.0042
folK	−	55.83	94.50	0.59	0.0066
queA	+	35.01	94.72	0.37	0.0002
folD	−	201.75	280.66	0.72	0.0001
cmoA	+	48.90	78.22	0.63	0.0032
cmoB	+	45.11	83.02	0.54	0.0014
folE	−	503.66	997.79	0.50	0.0006
folC	−	81.41	117.22	0.69	0.0001
glyA	−	1654.90	2405.51	0.69	0.0002
thyA	−	158.66	210.40	0.75	0.0036
gcvP	−	60.22	78.22	0.77	0.0155
metK	+	1287.57	1990.05	0.65	0.0009
folB	−	32.44	59.40	0.55	0.0074
folP	−	43.27	62.61	0.69	0.0093
tsaA	−	54.39	71.70	0.76	0.0020
sufZ(ygfZ)	+	352.50	316.19	1.11	0.0909
fau	+	63.27	45.84	1.38	0.0285
trmE	+	102.68	90.05	1.14	0.0865
metE	+	3672.39	12508.95	0.29	0.0103
metF	+	255.18	696.44	0.37	0.0003
folA	+	164.86	214.39	0.77	0.0234
gcvT	−	104.85	185.96	0.56	0.0048
mnmG(gidA)	−	90.74	129.82	0.70	0.0016
rlmF	+	33.44	70.17	0.48	0.0010
gcvH	−	162.36	312.22	0.52	0.0020
relE	−	347.26	185.79	1.87	0.0007
ssrA	+	366747.64	179404.45	2.04	0.0037
rimL	+	72.97	35.79	2.04	0.0056
dbpA	+	47.25	32.69	1.45	0.0094
rmf	+	33326.83	2402.80	13.87	0.0123
raiA	+	2099.84	1013.84	2.07	0.0186
rne	−	240.43	254.17	0.95	0.1211
rnd	−	78.16	69.95	1.12	0.1572
truD	−	215.05	221.30	0.97	0.2269
miaA	+	1078.36	988.68	1.09	0.2767
rluA	−	56.16	52.18	1.08	0.2841
arfA	−	16.65	14.52	1.15	0.4156
rluD	−	112.27	110.29	1.02	0.8154
truA	−	33.68	33.15	1.02	0.8534

Similar to the bulk of the genes related to translation, most of genes involved in 1-C metabolism showed lower expression (*P*-value < 0.05) during carbon limitation (grey background). In these previous experiments (Li *et al.*, 2019), gene expression was monitored by three independent RNAseq assays in a study investigating the behaviour of cells grown in a variety of carbon sources. Several examples of genes related to translation but showing higher expression (in bold) or no change (underlined) were also displayed for comparison [full data in Li *et al.* (2019)] The Rmf hibernation factor sequesters ribosomes away when translation has to slacken its pace.

a. Gene expression in carbon limited condition with a growth rate of 0.2 h^{-1}.

b. Gene expression in carbon-rich condition with a growth rate of 0.9 h^{-1}.

c. *p*-value was calculated using Student's *t*-test.

derived from other 1-C-dependent processes. As expected for the tRNA region responsible for translation accuracy, the main and most complex modifications occur at the anticodon loop of the molecule [Boccaletto *et al.* (2018) and Fig. 2].

General 1-C-dependent modifications

Overall, several methylases are conserved over a large number of bacterial clades (examples in Table S1). As discussed previously, TrmA is essential for viability in a way connected functionally to formylation of initiator methionine. The corresponding modification is, however, missing in many Tenericutes, which acquired small genomes from the Firmicutes by reductive evolution. This activity corresponds to a functional, not a metabolic, ubiquity of m^5U54: the methylation process differs in many Firmicutes, where it does not use AdoMet but CH_2-N^5,N^{10}-H_4F as further discussed below. Interestingly, the protein co-evolves with the gamma-proteobacteria degradosome (Engelen *et al.*, 2012), which may indicate that it has a role in protecting the tRNA molecule against degradation.

Besides formation of 5-methyluridine (m^5U, T), methylation processes result in the formation of many base modifications: 1-methyladenosine (m^1A), 5-methylcytidine (m^5C), methylation of position 1, 2 or 7 of G (m^1G, m^2G, m^7G), ribose 2'-O-methylation (Nm) and others [see Table 1 in Hori (2014)]. We document here only some of the most ubiquitous or significant ones (Fig. 2), noting that their nomenclature is unfortunately still quite variable. Briefly, two different roles are prominent for these modifications, a stabilizing role for the whole tRNA structure, sometimes involved in quality control of interaction with its cognate tRNA synthetase (Steiner and Ibba, 2019), and a role in the fine-tuning of the anticodon structure, directly related to the process of mRNA decoding in the ribosome. Extensive modifications are also important for tRNA maturation by RNase P, in particular in organelles (Karasik *et al.*, 2019).

The former category comprises a variety of functions stabilizing specific 3D structures of the tRNA molecule. For example, m^2G6 resulting from the action of methylase Trm14/TrmN stabilizes the amino-acyl stem in tRNAs present in organisms meeting desiccation or extreme conditions [*e.g. Deinococcus* sp. (Fislage *et al.*, 2012)]. The highly conserved G46 in the variable loop is methylated by methylase TrmB into modified m^7G46 that forms a tertiary base association with C13-G22. This stabilizes the tRNA structure and controls a hierarchy of modifications, in particular in thermoresistant bacteria (Tomikawa, 2018). A ribose methylation by TrmH at Gm18 stabilizes the interaction between the D-loop and the TΨC-loop (Ochi *et al.*, 2013). It may be required for import of tRNA into mitochondria (Paris and Alfonzo, 2018). Finally, a double methylation of base $m^2{}_2G26$ and also probably G27 corresponding to the generally unmethylated hinge region of *E. coli* $tRNA_{Phe}$ stablilizes the region in Aquifex or Pyrococcus families (Awai *et al.*, 2009; Sonawane *et al.*, 2016).

1-C-dependent modifications of the anticodon

Modifications of the anticodon, often derived directly from 1-C metabolism, have a critical role in the maintenance of translation accuracy and stability of the anticodon against RNases (Sokolowski *et al*., 2018). They are sometimes used for recognition of the cognate tRNA synthetase (Rodriguez-Hernandez *et al*., 2013). Decoding accuracy is illustrated by the role of m^6A37, due to adenine 37-N^6-methyltransferase TrmNF in $tRNA_{Val}^1$. Another function is illustrated by G37 N^1-methylase (TrmD), which is critical for multi-drug resistance (Masuda *et al*., 2019). In the yeast *Schizosaccharomyces pombe*, m^5C38 is methylated by DNMT2 (TRDMT1) methylase provided G34 has been replaced with queuine, a modified base that results from an atypical use of AdoMet [donation of its ribosyl – not its methyl – group (Johannsson *et al*., 2018; Muller *et al*., 2019)]. C38 methylation into m^5C38 is widespread and plays an important role in stress-related processes by protecting the anticodon from cleavage. In Eukarya, several enzymes are involved, in particular NSUN2 and DNMT2 (Gkatza *et al*., 2019). In the same way, Cm/Um34 methylation of the 2'-OH of cytidine/uridine 34 (C/U34m) by tRNA (cytidine/uridine-2'O)-ribose methyltransferase L (TrmL) requires the presence of the N^6-isopentenyladenosine A37 modification (i^6A37) for modification of Leu $tRNA_{UUR}$ necessary for correct RpoS sigma factor expression (Aubee et al., 2017). The ribose moiety at position 32 is also methylated, and Cm32/Um32 methyltransferase TrmJ is important for resistance to oxidative stress (Jaroensuk *et al*., 2016). The N32•N38 non-Watson–Crick anticodon pair is important for decoding, in particular decoding successive proline codons (Nguyen *et al*., 2019), while in some tRNAs, an A38 prevents incorrect methylation of U32 in the anticodon loop (Yamagami *et al*., 2012). tRNA methylation patterns display considerable variation, being particularly rich in Eukarya. The dialog between RNA modifications in the cytoplasm and in organelles parallels changes in 1-C metabolism in a fairly idiosyncratic way that cannot be further discussed within the scope of this article [for further references, see Burgess *et al.* (2015)]. Suffices it to remark that methylations are ubiquitously present, providing a powerful link between translation and 1-C metabolism.

In addition to methylation, and as in the case of ribosomal protein S12 modification, the anticodon of some tRNA molecules is thiomethylated. Spanning the whole three domains of life, this modification involves proteins MiaB or MtaB, depending on the organism of interest (Kang *et al*., 2017). MiaB is a bifunctional radical AdoMet iron–sulfur protein that participates in the methylthiolation of carbon 2 of the isopentenylated adenosine 37 residue, modifying tRNAs that read codons beginning with uridine (Hernandez *et al*., 2007). This modification is widely spread, being also present in animal cells where the reduction of its $[4Fe\text{-}4S]^{2+}$ cluster can be regulated by CysSSH, cysteine hydropersulfide, a mediator, the importance of which has only been recently acknowledged (Takahashi *et al*., 2017). In *E. coli*, multiple ferredoxins can also perform this redox reset (Arcinas *et al*., 2019). *In vivo*, MiaB acts together with a factor, SufZ (YgfZ), a still fairly enigmatic protein involved in iron–sulfur cluster synthesis and maintenance. Surprisingly, SufZ binds H_4F (Teplyakov *et al*., 2004), creating yet another link between folate metabolism and translation (Hasnain *et al*., 2012). Here, the role of H_4F is not entirely understood. It has been noticed that H_4F in homologous proteins may be used to remove formaldehyde from accidental adducts formed in iron–sulfur cluster enzymes (Teplyakov *et al*., 2004; Waller *et al*., 2010). Alternatively, in line with the activity of its homologue GcvT, SufZ might directly lead to CH_2-N^5, N^{10}-H_4F or, depending on the redox status of the catalytic environment, 1-C-H_4F derivatives of various oxidation states in a way reminiscent of demethylation of syringate (Masai *et al*., 2004).

It is further worth noticing that there is a counterpart of diderm SufZ in all domains of life, emphasizing the importance of the corresponding activity (Hasnain *et al*., 2012; Waller *et al*., 2012). For example, in yeast, an homologue, mitochondrial matrix protein YJR122w, is involved in incorporating iron–sulfur clusters into mitochondrial aconitase-type proteins (An *et al*., 2015). Importantly, SufZ is also involved in the zinc homeostatic response (Wu *et al*., 2009), a feature discussed in depth below. In terms of associated phenotypes, *sufZ*(*ygfZ*) null mutants grow poorly on minimal media, are hypersensitive to oxidative stress and, as just discussed, have reduced MiaB activity. As examined later on, the slow growth of *ygfZ* mutants was suppressed by an *mnmE* mutation, involving the regulation of yet another tRNA anticodon H_4F-dependent modification. Witnessing the importance of the integration of iron–sulfur clusters in the overall metabolism of the cell, the protein is involved, together with a ferredoxin, in the formation of membrane vesicles carrying toxins to their targets (Wang and Kim, 2013). SufZ is also required for the degradation of plumbagin – an herbal and carnivorous plant-derived toxic compound. *sufZ*(*ygfZ)* expression is induced by plumbagin, and *E. coli sufZ*(*ygfZ)* mutants are sensitive to the toxin (Chen *et al*., 2006; Lin *et al*., 2010). The 1-C metabolism is coupled to replication via thymine synthesis. As a further coupling between translation and replication, SufZ regulates the level of ATP-DnaA mediated by DnaA inactivator Hda (Katayama *et al*., 2017). This effect results from alteration of a tRNA modification (Ote *et al*., 2006). The involvement of Hda is consistent with the

inhibition of thymidylate synthesis by trimethoprim, an inhibitor of dihydrofolate reductase (Giroux *et al.*, 2017).

Finally, as yet another complex link with 1-C metabolism, it has recently been discovered that a 1-C modified variant of AdoMet, carboxy-*S*-adenosylmethionine, was necessary in many Gram-negative bacteria to modify base U34 of multiple tRNAs in their anticodon to convert 5-hydroxyuridine into 5-oxyacetyl-uridine at this wobble position [Byrne *et al.* (2013), several genes are involved in this process; see Fig. 1].

Folate-dependent modification of RNAs and proteins

At this point of our exploration, we have essentially taken into account the methionine/AdoMet-driven contribution of 1-C metabolism to translation. Yet, as discussed with the puzzling role of formylation of methionine-loaded initiator tRNA, further relevant tetrahydrofolate-mediated inputs of 1-carbon metabolites in the translation process are also prominent. A first hint of this role came from the alternative synthesis of methylated U54 in tRNA. Besides the action of TrmA in a large number of clades, this methylation involved CH_2-N^5,N^{10}-H_4F directly (Urbonavicius *et al.*, 2005). Furthermore, this original H_4F-dependent methylation was also observed in a ribosomal RNA modification in some organisms.

RlmFO and TrmFO

Indeed, the $FADH_2$-dependent, CH_2-N^5,N^{10}-H_4F-dependent enzyme encoded by Mcap0476 in *Mycolasma capricolum* was found to modify specifically base U1939 into m^5U1939 in 23S rRNA (Lartigue *et al.*, 2014), a conserved methylation catalysed by AdoMet-dependent enzymes in all other characterized bacteria (Danchin and Fang, 2016). This protein is a strict homologue of enzyme TrmFO that modifies position 54 in tRNAs of various microbial clades, indicating that the corresponding reaction has been propagated by horizontal gene transfer and adjusted to fit various RNA substrates.

To be sure, in many Gram-positive and some Gram-negative bacteria, thymine at position 54 is produced by a folate/FAD-dependent tRNA (m^5U54) methyltransferase [TrmFO (Dozova *et al.*, 2019)]. TrmFO utilizes CH_2-N^5,N^{10}-H_4F as a methyl donor and $FADH_2$ as a reductant. This remarkable coupling prevents oxidation of tetrahydrofolate to dihydrofolate during catalysis, as found in the widespread ThyA-dependent dUMP methylase that produces thymidylate for DNA synthesis. Moreover, in *Thermus thermophilus* cells, the m^1A58 modification forming a reverse Hoogsteen pair with U54 accelerates the TrmFO reaction, suggesting a synergistic effect of the m^5U54, m^1A58 and s^2U54 modifications on the complex m^5s^2U54 nucleotide formation in this akrophilic (extremophilic) organism (Yamagami *et al.*, 2012), and is consistent with an origin of the modification replacing the previous TrmA-mediated one. Overall, the presence of TrmA and TrmFO is mutually exclusive in the collection of genomes that are available to us. Interestingly, TrmFO belongs to yet another family of tRNA modification enzymes, the MnmG family that also uses H_4F derivatives to modify tRNA molecules (Urbonavicius *et al.*, 2005) and that we now discuss.

MnmE/MnmG/MnmC

The MnmEG complex modifies tRNAs decoding NNA/NNG codons in Bacteria and mitochondria (Armengod *et al.*, 2014). As does TrmFO, it modifies a uridine, but located at tRNA key position 34, the wobble anticodon position of many tRNAs, not position 54. This implies that the MnmEG complex must discriminate against a variety of uridine residues located within RNA loops. This discrimination is performed by the potassium-dependent GTPase activity of subunit E of the complex (Fislage *et al.*, 2016; Shalaeva *et al.*, 2018; Boel *et al.*, 2019; Danchin and Nikel, 2019; Gao *et al.*, 2019). Remarkably, this subunit also regulates the activity of the H_4F-related enzyme, poly-γ-glutamyl H_2F/H_4F synthase FolC, discussed at the beginning of this article. FolC is expressed in *E. coli* from a polycistronic *accD-folC-dedD* mRNA as a readthrough transcript of the monocistronic *accD* transcriptional terminator. FolC is further regulated by the glutamate/glutamine-sensing uridylyltransferase GlnD and UDP-glucose dehydrogenase Ugd. FolC inhibits the GTPase activity of MnmE at low GTP concentrations, further tying up folic acid metabolism to tRNA modification (Rodionova *et al.*, 2018).

Interestingly, another functional relationship links MnmEG with MiaB, discussed previously. Again, this goes via the activity of SufZ. Namely, deleting gene *mnmE* restores much of MiaB activity in a *sufZ* deletion strain, while overexpressing MnmEG exacerbates the growth and MiaB activity phenotypes of the *sufZ* mutant (Waller *et al.*, 2012). MnmG is involved in the transfer of a formaldehyde group to tRNA, in an H_4F-dependent process. This transfer is a source of metabolic accidents (Danchin, 2017b), as it may sometimes release this toxic metabolite locally but also possibly in the cytosol. Yet, plain formaldehyde release in the cytosol cannot explain the role of SufZ since a deletion of the *frmA* gene, coding for a glutathione-dependent activity detoxifying formaldehyde (Denby *et al.*, 2016), had little effect on growth or MiaB activity in a Δ*sufZ* strain grown in the presence of formaldehyde. However, this can be

accounted for. MnmEG might erroneously transfer a folate-bound formaldehyde unit to MiaB. Subsequently, SufZ might repair this accidental transfer (Waller *et al*., 2012).

Notably, MnmEG catalyses two different modification reactions, which add either a 5-aminomethyl (nm^5) or 5-carboxymethylaminomethyl ($cmnm^5$) group at position 5 of uridine 34 of certain tRNAs (Hagervall *et al*., 1987; Moukadiri *et al*., 2009). To this aim, the MnmE-MnmG complex uses CH_2-N^5,N^{10}-H_4F, $FADH_2$ and either ammonium or glycine as substrates (Ruiz-Partida *et al*., 2018). However, MnmEG can also modify all the relevant tRNAs while restricting its action to the ammonium pathway. In glutamine $tRNA_{cmnm5s2UUG}$ and leucine $tRNA_{cmnm5UmAA}$, $cmnm^5$ is the final modification, whereas in the remaining tRNAs, both MnmEG products are subsequently converted into 5-methylaminomethyl (mnm^5) via another enzyme, the two-domain, bifunctional methylase/oxidoreductase MnmC (Moukadiri *et al*., 2018). To perform these reactions, MnmC is both an AdoMet-dependent mnm^5s^2U34 methyltransferase and a FAD-dependent $cmnm^5s^2U34$ oxidoreductase (Kim and Almo, 2013). The two MnmC domains function independently of one another. Synthesis of mnm^5s^2U by MnmEG-MnmC *in vivo* avoids building-up intermediates in lysine $tRNA_{mnm5s2UUU}$. MnmE and MnmG, but not MnmC, are evolutionarily conserved.

Deletion of these proteins' genes affects the quality of the codon–anticodon interactions of the amino-acyl tRNAs with the mRNAs in the ribosome. Curiously, while these complex modifications are omnipresent, they are not strictly essential in laboratory conditions, suggesting that they manage transitions rather than unchanging conditions. Loss of MnmC activity has a biological cost in specific environments. To be sure, before these enzymes were identified, the inactivation of the corresponding genes was found to result in remarkable carbon source-related phenotypes (that account for the former nomenclature used to name them). MnmG was named GidA, for 'glucose-inhibited division protein A'. It was also shown to be involved in the development of fruiting bodies of bacteria such as *Myxococcus xanthus* (White *et al*., 2001), antibiotic production in *Pseudomonas syringae* (Kinscherf and Willis, 2002), quorum sensing in *P. aeruginosa* (Gupta *et al*., 2009) or toxin production in *Aeromonas hydrophila* (Sha *et al*., 2004). MnmE(GidB) was initially named ThdF and identified as a protein involved in thiophene oxidation (Alam and Clark, 1991), suggesting a further link between 1-C and sulfur metabolism. However, while the modifications were likely important for proper integration of metabolism, they did not usually have a significant effect on the growth rate of *E. coli* strains in standard growth conditions. In contrast, when the host strain was deficient in the synthesis of polyamines, deletion of the *mnmE* or *mnmG* gene resulted in complete inhibition of growth unless the medium contained polyamines (Rodionova *et al*., 2018; Shalaeva *et al*., 2018; Gao *et al*., 2019; Keller *et al*., 2019).

In summary, the role of H_4F-related modifications at base 34 of tRNA anticodons appears to coordinate the impact of various nutrient sources or physicochemical conditions experienced by the organisms as they transit through diverse environments and growth phases.

One-carbon metabolism and zinc assimilation and homeostasis

Reaching this point in our exploration, we did not find many compelling features that account for a tight functional link between 1-C metabolism and translation, except as a frozen accident of prebiotic metabolism. Yet, perhaps, this link might witness a buffering role in protecting the cell against what we might name 'reactive one-carbon species' (ROCS) via processes scavenging those, especially formaldehyde. This may underpin the role of the enigmatic SufZ(YgfZ) protein. Carbon input *via* H_4F into an active 1-C metabolism involved in a variety of translation-related substrate modifications provides clues to identify further relevant processes. Nevertheless, besides the protective role related to the carbon flow, two features kept appearing in our quest: iron–sulfur cluster management and potassium-dependent activities, possibly connected to nitrogen metabolism *via* ammonium availability [ammonium and potassium have common physicochemical features, discussed in Danchin and Nikel (2019)]. Both involve omnipresent metal ions. Surprisingly, a third metal, zinc, now enters the picture and this may reveal the key to spot the most significant link between 1-C metabolism and translation.

The cell's life is entirely dependent on divalent metals that play critical roles in generalized acid catalysis, maintenance of the local electric charges or electron transfers. Among those, zinc, under the Zn^{2+} form, is universally present, presumably because of its unusual physicochemical properties when interacting with water and proteins, in particular its coordination sphere that most often is not octahedral (Vahrenkamp, 2007). Yet, this metal competes with other ones [mainly Mg^{2+} or Ca^{2+}, besides Fe^{2+} and Mn^{2+}, or copper, nickel and cobalt (Xu *et al*., 2019)], so that its concentration has to be maintained within very narrow borders to prevent interference with the function of these other metals. This need asks for a storage/buffering system (Takahashi *et al*., 2015). Thiols, in the form of glutathione or bacillithiol, appear to play a key role in serving as major buffers of the labile zinc pool (Ma *et al*., 2014; Krezel and

Maret, 2016). Still, these versatile protective compounds are also involved in a large number of other safeguard roles – buffering formaldehyde is highly relevant in the present context (Vorholt, 2002; Muller *et al*., 2015; Chen *et al*., 2016) – so that their capacity to regulate the zinc pool remains limited, being challenged by frequent changes in the redox level or by the omnipresence of reactive aldehydes (see discussion below).

This remark leads us to contemplate another key feature of 1-C metabolism: 1-C-H_4F precursors are required in the synthesis of purines. To be sure, purine *de novo* biosynthesis uses, twice, 1-C donors in the form of CHO-N^{10}-H_4F besides three amino acids, glutamine (twice), glycine and aspartate. It has long been known that a low intracellular concentration of CHO-N^{10}-H_4F leads to accumulation of the intermediate 5'-phosphoribosyl-4-carboxyamide-5-aminoimidazole (AICAR, alternatively named ZMP, also a side product in histidine salvage/synthesis), which is subsequently pyrophosphorylated to alarmone ZTP by an unidentified enzyme [(Bochner and Ames, 1982), but see Rohlman and Matthews (1990)]. That ZMP/ZTP (Z nucleotides) are authentic regulators of gene expression is supported by the presence, in a variety of bacterial clades, of Z riboswitches involved in the control of purine but – this is highly relevant here – also of folate biosynthesis (Greenlee *et al*., 2018). Unexpectedly, recent studies in *B. subtilis* have linked these metabolites to translation, not directly but via the metabolism of an essential metal, zinc. As an epiphyte, this organism meets extremely variable environments, in particular in the phylloplane, where the availability of many divalent ions is limited (in unpolluted environments) and must display fine regulation of their availability in the cell. Because purine biosynthesis rests on zinc-dependent enzymes, any zinc limitation affecting H_4F synthesis tends to accumulate Z nucleotides. In *B. subtilis*, this triggers a sequence of events where the transcription factor Zur-regulated metallochaperone ZagA (YciC) is activated by ZTP to deliver zinc to FolE, the first enzyme of the pathway to sustain folate synthesis (Chandrangsu *et al*., 2019; Zhang *et al*., 2019). This creates a tight connection between the synthesis of this alarmone, H_4F and the level of the Zn^{2+} ion in cells. When cells are facing the presence of antifolic drugs, this nexus should be revealed. Alas, early experiments – where the Zn^{2+} concentration was not controlled – failed to realize this direct connection between Z nucleotides and folic acid metabolism (Rohlman and Matthews, 1990), and this explains why it took so long to be appreciated.

This recent set of experiments paves the way to understand this critical network of interactions. In *B. subtilis*, H_4F synthesis – beginning with GTP – is split into two pathways, depending on Zn^{2+} availability. In contrast to the widespread situation where the first step of the pathway producing the 1-C metabolite formate and 7,8-dihydroneopterin-3'-triphosphate is catalysed by a single Zn^{2+}-binding GTP cyclohydrolase 1, FolE, *B. subtilis* accommodates a zinc-independent counterpart, FolEB, which is activated when Zn^{2+} becomes strongly limiting. In parallel, the zinc GTP-dependent metallochaperone ZagA responds to ZTP availability and supports *de novo* folate biosynthesis by interacting directly with the zinc-dependent GTP cyclohydrolase IA, FolEA (Shin and Helmann, 2016; Chandrangsu *et al*., 2019). ZagA seems to deliver Zn^{2+} to FolEA directly to sustain this Zn^{2+}-dependent GTP cyclohydrolase as the level of Zn^{2+} drops. This binding interaction is activated by ZTP, transiently accumulating as FolE begins to fail, establishing the role of ZagA as a sensor of CHO-N^{10}-H_4F deficiency in bacteria. Other types of transition metals with dissociation constants in the low micromolar range also affect both the oligomeric structure and GTPase activity of the enzyme. Being responsible of discrimination of Zn^{2+} against other divalent cations, ZagA could thus be yet another example of a key informational GTPase (Boel *et al*., 2019).

How does this intricate process connect to translation? Prominently, the ribosome appears to act as the main cellular Zn^{2+} store. To be sure, homeostatic regulation of Zn^{2+} availability develops in parallel with alteration of the translation machinery, via neosynthesis of three zinc-free ribosomal proteins, L31 (RpmEB) and L33 (RpmGC) directly, together with S14(RpsNB) that acts as a zinc buffering protein (Nanamiya and Kawamura, 2010). As a consequence, translation keeps remaining active as long as possible while the zinc supply becomes depleted.

Is this general? In *E. coli*, the response to zinc limitation has been explored in considerably less detail than in *B. subtilis*, but it is still revealing. In this organism, there is only one FolE enzyme, which is Zn^{2+}-dependent. However, GTPase ZinU(YjiA) may play a role similar to that of ZagA (Nies, 2019). *E. coli* also comprises another metal-binding GTPase, YeiR, that may have its activity stimulated upon physical, even transient, association with an apoenzyme target or client protein, thus providing a driving force for intermolecular Zn^{2+} transfer (Blaby-Haas *et al*., 2012). In line with a variety of counterparts, this protein contains a CXCC motif that, upon relevant metal binding, is likely to trigger its GTPase activity (Jordan *et al*., 2019). This overall similarity between distant organisms is further extended to the connection with translation: *E. coli* also codes for alternative ribosomal proteins that lack a zinc-binding cysteine cluster and are expressed under Zn^{2+} limitation, namely L31, RpmEB(YkgM), and L36, RpmJB(YkgO) (Graham *et al*., 2009; Hensley *et al*., 2012; Gutierrez-Huante *et al*., 2019). Remarkably, a shift from the Zn^{2+}-replete ribosome to the deficient ones occurs upon transition from the exponential to the stationary growth

phase (Lilleorg *et al.*, 2019). This parallels the process of Zn^{2+} deficiency-induced hibernation in *Mycobacterium tuberculosis* (Li *et al.*, 2018).

Witnessing its important role as a zinc buffering system, this coupling between zinc availability and translation is widely spread, with ribosomal proteins comprising zinc-finger motifs distributed everywhere in the tree of life (Kovacs *et al.*, 2018). However, a similar role in Eukarya has not yet been explored, while ZMP has essentially been identified as an activator of the crucial AMP-activated protein kinase (Camici *et al.*, 2018). Furthermore, many zinc-binding proteins are involved in the control of translation in the organisms of this domain [see, e.g. Kovacs *et al.* (2018) for ribosomal proteins analogues of the bacterial counterpart or Yonezawa *et al.* (2014) and Sloan *et al.* (2019) for other relevant translation-related processes]. Hence, the connection between 1-C metabolism, translation and zinc homeostasis is likely to be common to all three domains of life. The details and functional rationale of the corresponding networks remain, however, to be deciphered. A starting point for experimental exploration of this crucial role of zinc might use gliotoxin, a mycotoxin likely to act as a zinc scavenger, a pseudargyrophore [to use the Greek root for zinc, see Iliopoulos *et al.* (2019)] as a way to modulate zinc availability, translation and the overall energy metabolism (Seo *et al.*, 2019).

Phylogeny of some folate-dependent enzymes involved in translation

Most of the previous discussion argues that 1-C metabolism, whether *via* folic acid, methionine or AdoMet, was associated with early steps of the emergence of the translation process. It is therefore of interest to investigate how extant structures of some of the cognate enzymes are phylogenetically related to each other. At this point, the question arises about the origin of the various processes that connect translation to 1-C metabolism. It is generally accepted that invention of DNA was a late discovery in the emergence of the first cells, resulting in the stabilization of a vulnerable RNA-based metabolism and RNA-genome replication. Within this scenario, RNA modifications were developed prior to DNA synthesis, namely prior to thymine discovery. Thymine synthesis could thus be used as a baseline in the present investigation. Two H_4F-dependent pathways result in thymine synthesis, using either ThyA or ThyX enzymes. Their phylogenetic relationships have been explored, and the conclusion reached is that their distribution in extant living organisms is heavily dominated by the process of horizontal gene transfer [(HGT) (Stern *et al.* (2010)]. Yet, because ThyA uses only one substrate, H_4F, besides dUMP (Fig. 1), it seems likely the first enzyme to emerge in the process. Indeed, its anabolic role rests on the presence of dihydrofolate reductase (DHFR), which must have already been present as a key enzyme allowing the synthesis and maintenance of a reduced pool of pterin and folate derivatives. Evolution experiments have explored in *E. coli* the fate of genes transferred by HGT when a DHFR gene is replaced by a foreign one. Remarkably, regaining full efficiency of the foreign enzyme in its new context required many generations, in particular in order to overcome the action of the 'self'-discriminating ATP-dependent protease Lon (Bershtein *et al.*, 2015; Boel *et al.*, 2019). Multiple HGT events would blur the picture even further. As a consequence, trees with reliable rooting could seldom be safely constructed when HGT is involved in the processes that led to the presence of any extant gene.

As another way to infer possible origins of the links that associate 1-C metabolism and translation, we may explore the phylogeny of the enzymes that allow biosynthesis of folates and that of methionine synthase – which is Zn^{2+}-dependent. A general study, focused on plants but with some comparisons with *S. cerevisiae* and *E. coli* and animals, showed a variety of trees, which, again, would lend credence to widespread HGT, precluding faithful identification of relevant rooting (Lian *et al.*, 2015). In this context, the recent identification of a methyl-B12-dependent, H_4F-independent form of the enzyme should be interpreted as a recent acquisition (synthesis of coenzyme B12, requiring at least 30 genes, is hardly an early feature of life), rather than primitive (Deobald *et al.*, 2020).

As discussed previously, a double origin of thymine is also observed for modification of uracil 54 into thymine (m^5U54) in tRNAs. It does not depend, however, solely on H_4F metabolism. This modification, performed either by AdoMet-dependent TrmA or by H_4F-dependent TrmFO, is concomitant with that of rotation of the uracil base of uridine 55 into a pseudouridine base by the TruB(Pus4) family of pseudouridylate synthases. In many Archaea, a novel pseudouridylate synthase, Pus10, produces Ψ55 and Ψ54, which then take the place of T54 (Gurha and Gupta, 2008). While there is significant similarity between eukaryal (*H. sapiens*) and archaeal (*M. jannaschii*) Pus10 orthologues, no similar protein sequences were found in Bacteria. With the present available sequence data, it appears that that only TruB and TrmA orthologues remained functionally unchanged. Pus4 (TruB orthologue) converts U55 to Ψ55, and Trm2 (TrmA orthologue) modifies U54 to m^5U54 in tRNAs of eukaryotes (Fitzek *et al.*, 2018). TrmA and TruB are absent in Archaea, but are present in Bacteria and Eukarya. This parallels the presence of *sn*-glycerol-1-phosphate in the membrane phospholipids

of the former and *sn*-glycerol-3-phosphate in those of the latter, substantiating an important common origin between Eukarya and Bacteria. In the same way, the analysis using EggNog (Huerta-Cepas *et al.*, 2019) exploring the relationships between MnmG(GidA) sequences – that are related to TrmFO – showed that it could well be rooted in ancestors of Eukarya, suggesting a very ancient origin (tree displayed at http://eggnogdb.embl.de/#/app/results#COG0445_datamenu). This was again consistent with an origin based on large partially differentiated populations of cells where HGT was pervasive (Doolittle and Brown, 1994; Kim and Caetano-Anolles, 2011; Fournier *et al.*, 2015).

Using EggNog again, similar conclusions can be reached when exploring the phylogenetic relationships between SufZ(YgfZ) proteins (tree displayed at http://eggnogdb.embl.de/#/app/results#COG0354_datamenu). However, in Eukarya, these proteins appear to be located within organelles, so that the actual order of emergence of these very early functions cannot be firmly established. In general, the enzymes involved in tRNA modifications in Archaea tend to differ both from those of Eukarya or Bacteria, consistent with a fairly separate set of constraints in the metabolism of Archaea that allowed them to split from the original cell populations.

Perspectives

Methionine and AdoMet are the main output of folic acid-mediated 1-C metabolism. This is consistent with folate metabolism being primaeval, parallel with purine, flavin and pterin biosyntheses in a general GARD-like process that created an opportunity for nitrogen fixation (Danchin, 2017a; Lancet *et al.*, 2018). The way we appraise metabolism will heavily depend on preconceived ideas about the nature of the first cells. For example, if Bacteria were primitive, then formylation of the first methionine of polypeptide would probably be a primitive feature (Di Giulio, 2001). In contrast, if the metabolic origin of proteins was distributed within large populations of cells that kept exchanging

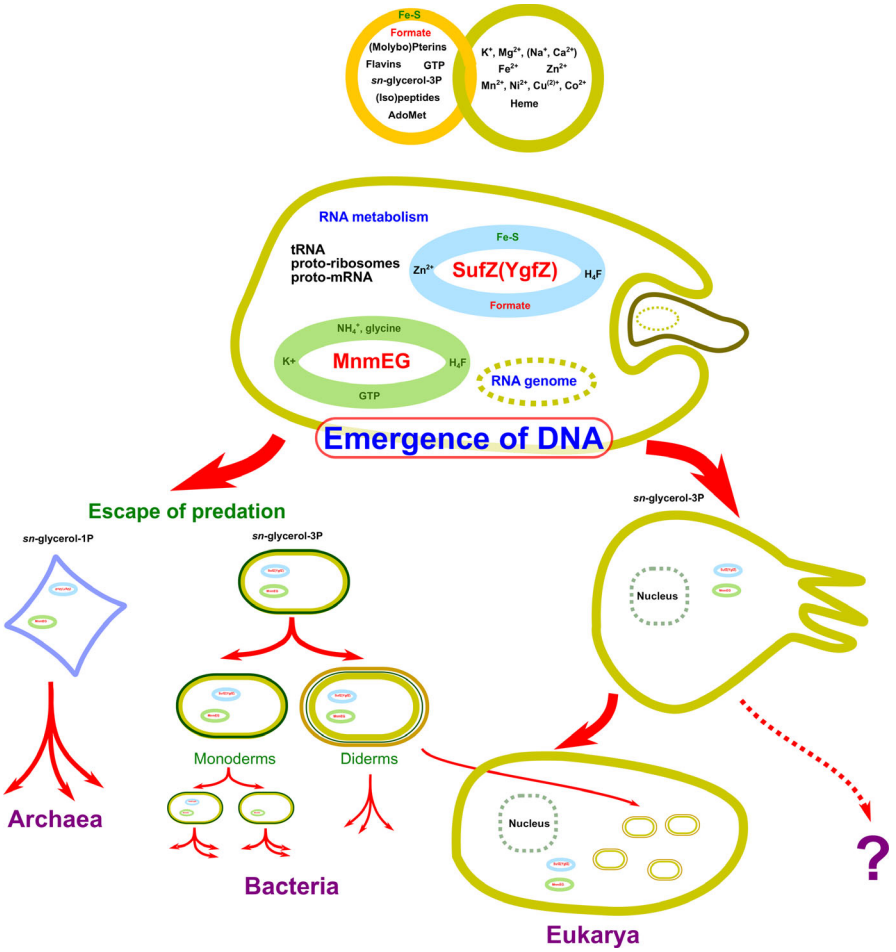

Fig. 3. One-carbon metabolism from the first cells to extant metabolism. Here, we follow Charles Kurland's view of evolution from predator cells to the current three domains of life (see text). The MnmEG-SufZ network of tRNA modifications integrates translation with 1-C metabolism and Zn^{2+}-controlled Fe^{2+}-S cluster maintenance and insertion into polypeptides.

metabolic pathways and primitive genetic set-ups, then we have to take into account the logic of metabolism, trying to put together pathways that would fit with Okham's razor constraints. Here, we favoured the scenario proposed by Charles Kurland where primitive cells were fairly large scavenger organisms (not an universal last ancestor – UCA, but a last ancestral cell ensemble – LACE) in a predator/prey dialog. This created a novel function, that of evading predation, which ended up with streamlining compartmentalization as prokaryotes progressively became smaller, with Bacteria having a solid 'uneatable' envelope, while Archaea used a different stereochemistry for the phospholipids of their membranes, making predation difficult or sterile [Fig. 3; see https://www.youtube.com/watch?v=3iD0RiNw9B4 and Danchin (2017a)]. In this scenario, H_4F (and other pterins), formate and nucleotides were primitive compounds. They were linked to metabolic pathways involving iron–sulfur clusters, followed by emergence of methionine and AdoMet, then RNAs and RNA metabolism. A crucial step at this early stage was the RNA-dependent formation of polypeptides that ended up in the process of translation.

Do we have evidence of integration of the metabolic processes tied up to translation as displayed in Fig. 1? To be sure, in extant organisms this relationship appears to be substantiated. Remarkably, the expression of most of the genes involved in the 1-C processes associated with translation is indeed varying in a concerted fashion, which differs from that of processes involving other metabolic pathways. As shown in Table 1, the genes displayed in Fig. 1 are expressed in *E. coli* – certainly a highly evolved organism – in parallel. They collectively displayed lower expression when the growth rate was reduced during carbon limitation, as does the bulk of the translation machinery (Li *et al*., 2019). However, as displayed in the table, this differs from the behaviour of other genes involved in translation but not related to 1-C metabolism, which either does not vary or displays an increased expression. Besides tRNA modifications not stemming from 1-C metabolism such as MiaA or TruA that follow the general expression of the translation machinery, the behaviour of ribosomal protein L12 serine *N*-acetyl-transferase RimL – hence involving 2-C metabolism – is a case in point as it is enhanced rather than being repressed. This is an indirect proof of the functionally significant interconnection between 1-C metabolism and translation, because no specified involvement of 1-C metabolism was input in the way carbon-sensitive gene expression was measured in those experiments (Li *et al*., 2019). The fact that we made this observation in an extant organism may reflect an early state of metabolism that has been maintained because of its selective value. This supports a functional – not accidental – link between 1-C metabolism and translation. However, this does not directly tell us what could be the corresponding functional advantage. We now propose some tracks to understand this function/ metabolism coupling.

A first functional pressure must be connected to the universal presence of a methionine residue as the first residue of all polypeptides. As suggested previously, this could reflect the need for a highly flexible hydrophobic residue to allow translation initiation to proceed smoothly. Indeed, initiation of translation is an asymmetric process that begins at the peptidyl site of the ribosome, binding a tRNA with a different conformation of the anticodon – where base 36, not 34, is subject to wobbly recognition by the start codon – not at the A-site as other loaded tRNAs subsequently bind. Substantiating the importance of this mechanical step is the fact that, in Bacteria, formylation of the first methionine is used in polycistronic operons to make the 70S ribosome ready for initiation, without requiring its recycling into dissociated 50S + 30S subunits (Qin *et al*., 2016). This function appeared as a consequence of the clustering of genes into operons that took place progressively as natural selection accommodated the coexpression of related functions. To be sure, the chromosome set-up of the Bacteria domain has been constrained by the importance of genes that are critical for survival ['persistent' genes (Fang *et al*., 2008)] providing a selective pressure for the omnipresent formation of operons. Yet, the formylation process could only be fully developed after translation had already accommodated proline into polypeptides [with recruitment of a specific elongation factor, EF-P (Katoh *et al*., 2016)]. Taken together, these features are consistent with fMet at the protein start as a recent acquisition [rather than a primitive feature as proposed by (Di Giulio, 2001)], further tying up translation with 1-C metabolism. Importantly, this supports an origin of Bacteria later than the LACE (Fig. 3). That formylation has only an accessory role, which could not be the main driving force for the coupling of 1-C metabolism and translation, is further supported by the observation that translational coupling in operons is essentially based on the presence of a significant ribosome binding site in the upstream gene sequence (Huber *et al*., 2019).

In Eukarya – derived from LACE-compartmentalized predators after Bacteria had been engulfed as symbiotic organisms – folate metabolites are compartmentalized, with up to half of the folate residing in the mitochondria and the remainder in the cytoplasm. Folate-dependent reactions are catalysed by two distinct groups of enzymes, cytosolic and mitochondrial. Some folate-dependent enzymes are present in both compartments. Formylation of initiator methionine in mitochondrial proteins appears to be a way to export 1-C compounds, redistributing 1-C metabolites in the cell. As previously discussed, an important associated function is disposal

of ROCS. A further substantiation of this view is the role of a cytosolic H_4F-dependent enzyme, CHO-N^{10}-H_4F dehydrogenase (ALDH1L1), which contains a domain with significant sequence similarity to aldehyde dehydrogenases. This domain enables catalysis of the $NADP^+$-dependent conversion of short-chain aldehydes to corresponding acids *in vitro*. The reaction is the final step by which an H_4F-bound formyl group is oxidized to CO_2 in a $NADP^+$-dependent fashion, thus alleviating the toxic action of ROCS. A mitochondrial counterpart, ALDH1L2, arose from a duplication event of the ALDH1L1 gene prior to the emergence of osseous fish> 500 millions years ago (Strickland *et al*., 2011), extending this detoxification process to mitochondria. In conclusion, a selective pressure for maintaining an early coupling between 1-C metabolism and translation in Eukarya could be mitochondria-based detoxification of ROCS.

A further universal role of 1-C metabolites in translation stems from AdoMet-dependent multiple methylations of ribosomal RNA and tRNA. Many of the modifications are essential and were certainly present during the times of the RNA-metabolism and RNA-genome worlds. Another universal methylation step, methylation of release factors terminating translation, is also essential. Finally, a completely different function might lie in the unusual AdoMet-dependent modification of the G34 base position in the anticodon of some tRNAs. There, control of translational speed and accuracy to manage polypeptide folding would be fine-tuned by availability of the microbiologically derived queuine metabolite (that exchanges for G34), coupling nutrition with endogenous microbiota (Tuorto et al., 2018) and providing yet another link between 1-C metabolism and translation (Fig. 1). All these modifications create a strong link, coupling methionine – hence sulfur metabolism – to the input of prosthetic groups – mainly iron–sulfur clusters – within polypeptides.

Yet, the most intriguing role of 1-C metabolism – possibly accounting for its omnipresent connection with translation – is that played in the homeostasis of Zn^{2+}, where ribosomes act as the main Zn^{2+} store (Hensley *et al*., 2012). In *B. subtilis* – we have to wait for new experiments to see how general is this feature – a sophisticated regulatory circuit that involves the alarmone ZTP was recruited to optimize cellular Zn^{2+} distribution when the ion becomes limiting (Nies, 2019). As described previously, this process uses zinc-dependent enzymes to probe the pool of available Zn^{2+} ions, and then amplifies this signal to control the activity of Zn^{2+} chaperones. It drives biosynthesis of purine, folate and 7-deazaguanine – the precursor of the queuine residue replacing G34 is some tRNAs, not only in *B. subtilis* but also in other bacteria (Sankaran *et al*., 2009). While the whole of this pathway is not strictly ubiquitous – many animals do not synthesize H_4F *de novo*, for example – the first step mediated by GTP cyclohydrolase and essential for neuromediators biosynthesis remains Zn^{2+}-dependent. Further, the fact that many zinc-dependent enzymes that are required to allow translation to proceed – with tRNA modifications as substantiating evidence in all domains of life – are directly dependent on 1-C metabolism strongly argues for a functional dependency relating translation, 1-C metabolism and zinc homeostasis, possibly via management of iron–sulfur clusters. Fig. 1 summarizes the sophisticated interaction network that ties together translation and the 1-C metabolism. The key metabolites of this network are formaldehyde, methionine, S-adenosylmethionine and tetrahydrofolate. A triad of tRNA U34 modification enzymes, namely MnmE, MnmG and SufZ, appear to create a nexus of interactions between translation, folic acid metabolism, ROCS, management of iron–sulfur clusters and zinc availability, with the latter playing the role of general coordinator. This set of tRNA modifications is further used for phosphate homeostasis by thiolation of the same U34 base (Gupta and Laxman, 2019), making this position ideal to balance carbon, nitrogen, sulfur and phosphate metabolism via a Zn^{2+}-mediated translation control of Fe^{2+}-S cluster synthesis, input into polypeptides during translation and maintenance.

Finally, a noteworthy 1-C metabolic feature must be taken into further consideration. In a great many cells, methionine synthesis requires the presence of coenzyme B12 derivatives (cobalamins). This is because methylation of homocysteine is very inefficient in the absence of cobalamins, requiring a large amount of the corresponding enzyme (MetE in Bacteria). In Eukarya, there is elevated formate in vitamin B-12 deficiency with concomitant creation of deleterious ROCS, with detoxification, as discussed previously, via CO_2 production. Formate is produced in mitochondria via the catabolism of serine, glycine, dimethylglycine and sarcosine. This compound may be incorporated into the cytosolic folate pool where it can be used for important biosynthetic reactions. During cobalamin deficiency, the fate of CHO-N^{10}-H_4F carbon is shifted in favour of formate production. This may represent a mechanism to generate more one-carbon units for the replenishment of the AdoMet pool, which is depleted in this condition (MacMillan *et al*., 2018), but this is at the cost of ROCS production. Remarkably, recent studies have identified many more enzymes binding B12 derivatives than the usual B12-dependent ones [methionine synthase primarily, but many other ones as well, including methylases (Sankaran *et al*., 2009)]. In particular, several enzymes involved in queuine biosynthesis have been found either to require B12 [QueG (Dowling *et al*., 2016)] or to bind to it [QueA (Romine *et al*., 2017)]. This creates an additional link between translation and 1-C

metabolism, suggesting that there is indeed an important functional requirement involving this interaction. Requiring more than 30 genes needed for their synthesis (Fang *et al*., 2018), cobalamins are latecomers in the panoply of coenzymes. The fact that B12-dependent methionine synthase and queuine biosynthetic enzymes kept involving zinc in their activity is yet another substantiation of the role of Zn^{2+} in integrating the process of translation, 1-C metabolism and general metabolism.

Conclusion

To complete this overview, let us stress again in the end that, in addition to tying up translation to 1-C metabolism, H_2F/H_4F-dependent synthesis of thymidylate creates a tight coupling between translation and replication. To be sure, the consumption of CH_2-N^5,N^{10}-H_4F by TrmFO has a negative effect on dTMP and methionine syntheses. This results in slow growth under nutrient-poor conditions (Yamagami *et al*., 2018). These metabolic features can be organized into a general picture, suggesting that the importance of 1-C metabolism was already dominating primaeval cells' metabolism. Furthermore, the collection of data that puts together molecules that were linked to this metabolism can be best interpreted as involving RNA molecules, in particular the ancestors of tRNAs as substrates of a large number of metabolic pathways. This would have been present at a time when the ribosome was a peptide forming nanomachine, initially linking amino acids together in a more or less random fashion. When 'gene RNAs' began to play the role that messenger RNAs have today, further modifications of tRNAs became useful or even indispensable. Finally, the processes required to construct enzymes had to couple translation with insert of prosthetic groups, iron–sulfur clusters in particular, and control of this process by Zn^{2+} availability, stored by ribosomes, would have created a remarkable opportunity to fulfil this function.

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Conflict of interests

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Important methyltransferases involved in translation.

