Minireview

Transfer RNA modification: influence on translational frameshifting and metabolism

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Abstract Transfer RNA modification improves the rate of aa-tRNA selection at the A-site and the fitness in the P-site and thereby prevents frameshifting according to a new model how frameshifting occurs [Qian et al. (1998) Mol. Cell 1, 471–482]. Evidence that the presence of various modified nucleosides in tRNA also influences central metabolism, thiamine metabolism, and bacterial virulence is reviewed.

Key words: tRNA; Modified nucleoside; Frameshifting; Central metabolism; Virulence

1. Introduction

Transfer RNA from all organisms contains modified nucleosides and so far 79 have been characterized [1]. Some modified nucleosides, such as 1-methylguanosine (m^1G37), are present in a specific position in a subset of tRNAs from all three domains – Archaea, Bacteria, and Eucarya – suggesting that they were present in the tRNA of the progenote [2,3]. Other modified nucleosides are present in only two domains whereas some are domain specific [4]. It has been estimated that about 1% of the genetic information present in bacteria is devoted to enzymes catalyzing the formation of modified nucleosides [5]. Surprisingly, this is four times more than what is required to make all the various tRNA species [5]! Although the function of modified nucleosides has for many years eluded the scientists, we now know that they improve the fidelity and efficiency of tRNA in decoding the genetic message and by maintaining the reading frame (reviewed in [6]). The environment affects the degree of tRNA modification, which in turn influences translation and thereby it may affect central and intermediary metabolism [7]. The influence of tRNA modification on reading frame maintenance, on central and intermediary metabolism, and on the virulence of a bacterium will be reviewed.

2. Transfer RNA modification and translational frameshifting

Most missense errors resulting in amino acid substitutions do not profoundly influence the function of a protein. However, reading frame errors are more detrimental, since such errors result in the synthesis of an erroneous and most often an incomplete peptide. Therefore, the prevention of translational frameshifting by modified nucleosides may be more important for the survival of the cell than their prevention of missense errors.

Frameshift mutations are of two types: an addition of a nucleotide (+1 frameshift mutation) or a deletion of a nucleotide (−1 frameshift mutation). Extragenic mutations that suppress +1 frameshift mutations were shown to result in derivatives of either $tRNA^{Gly}$ or $tRNA^{Pro}$, suppressing at GGG-N and CCC-N sites, respectively (+1 frameshift occurs when the ribosome moves from e.g. GGG to the GG-N where N can be any nucleotide)(reviewed in [8]). +1 suppressor mutants of $tRNA^{Gly}$ have an extra C in the anticodon loop [9,10], whereas +1 suppressor mutants (*sufA6* and *sufB2*) of $tRNA^{Pro}$ have an extra G in the anticodon loop [11,12]. Based on these observations an elegant and simple model was suggested explaining how these mutant tRNAs suppress +1 frameshift mutations: a four base anticodon base-pairs in the A-site with four bases in the mRNA and following a quadruplet translocation, reading in the zero frame is regained [9]. However, this model is inadequate to explain the action of several +1 frameshift suppressor tRNAs with a normal sized anticodon [11–15]. Surprisingly, it was shown that the frameshifting event induced by one of the classical suppressors, *sufB2*, occurred after translocation into the P-site [16]. In addition, the classical frameshift suppressors, *sufA6* and *sufB2*, were shown to have the extra G inserted 3′ of m^1G37. Since m^1G37 prevents base-pairing with C [17], this position of the extra G creates a three nucleotide anticodon bordered on the 5′ side by U33 and on the 3′ side of m^1G37, clearly invalidating the model at least for these two classical suppressors [18]. Moreover, in the presence of *sufB2* $tRNA^{Pro}$, the frameshifting event in the P-site is exerted by a wild type near-cognate tRNA instead of the mutant *sufB2* tRNA [18]. Thus, these lines of evidence suggest that the classical frameshift model including a quadruplet translocation from the A-site to the P-site is not correct. Therefore, we have suggested a new model explaining how most, and perhaps all, +1 and −1 frameshifts occur [18].

According to the dual error model (Fig. 1), shortage, or slow entry, of an aa-tRNA into the A-site induces a pause during which a near-cognate tRNA is accepted (first error). Following a normal three nucleotide translocation the near-cognate tRNA slips into the +1 or −1 frame due to an aberrant anticodon-codon interaction in the P-site (second error). Alternatively, an altered tRNA, e.g. a modification deficient tRNA, may be accepted into the A-site instead of a near-cognate tRNA. After a normal translocation, this tRNA

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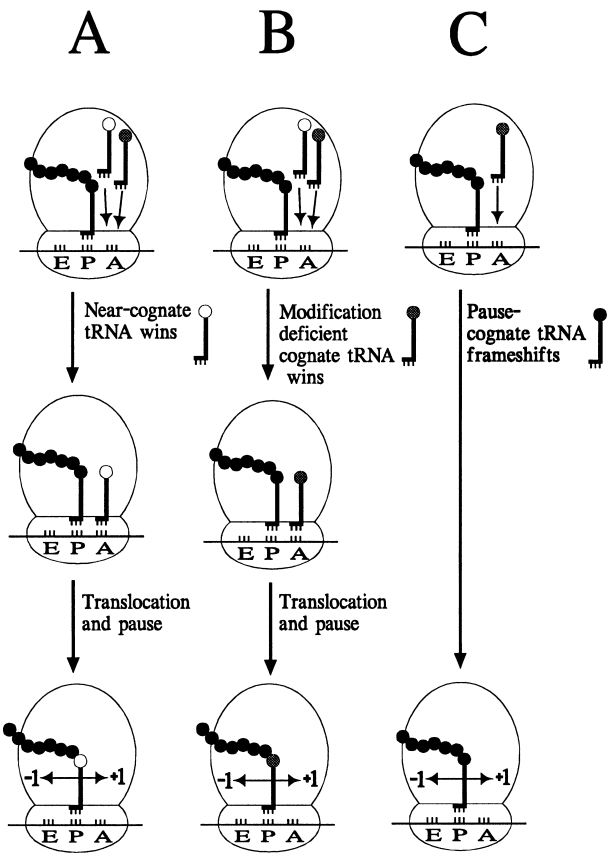

Fig. 1. The dual-error model for frameshifting. A: Hypomodified cognate tRNA (gray circle on tRNA) is defective in the aa-tRNA selection step and thereby allows a wild type near-cognate tRNA (white circle on tRNA) to be accepted instead at the A-site. After a normal three nucleotide translocation, the near-cognate tRNA slips into either −1 or +1 frame depending on the sequence of the mRNA. B: As in A but the hypomodified cognate tRNA is accepted in the A-site and when residing in the P-site, the hypomodification induces slippage into the −1 or +1 frame. C: The hypomodified tRNA is very slow in entering the A-site inducing a pause and allowing the wild type tRNA (black circle on tRNA) already in the P-site to frameshift.

may be prone to frameshift in the P-site [18,19]. This new model predicts that modification deficient tRNA may influence reading frame maintenance at two steps during the translation cycle. (1) In the A-site: an undermodified cognate tRNA may be slow entering the A-site and thereby triggering a near-cognate tRNA to be accepted (Fig. 1A). Following a normal translocation to the P-site, this near-cognate tRNA may be prone to frameshifting in the P-site. If a long pause is induced by the undermodified tRNA and if no near-cognate tRNA can compete, the wild type tRNA already in the P-site may shift frame (Fig. 1C). (2) In the P-site: if the hypomodified tRNA is accepted at a reasonable rate at the A-site, a three nucleotide translocation moves it into the P-site where frameshifting may be induced (Fig. 1B). We have earlier shown that m^1G37 deficiency reduces Pro- and Arg-tRNA selection at the A-site (Fig. 1A) but not the selection of Leu-tRNAs [20]. Moreover, for some tRNA species, ms^2io^6A37, mnm^5s^2U34, or Q34 deficiency reduces the rate of selection at the A-site (Fig. 1A,C). Interestingly, lack of ms^2io^6A37 in $tRNA^{Phe}$ does not influence the rate of selection at the A-site either in vitro [21] or in vivo [20]. However, $ms^2i(o)^6A37$ deficiency induces frameshift in the P-site provided that the ribosome pauses for other reasons such as shortage of the next A-site aa-tRNA or presence of a termination codon in the A-site [22,23] (Fig. 1B). Of course, modified nucleosides, which influence the reading frame maintenance by improving the A-site selection, may also prevent slippage in the P-site. Clearly, the presence of modified nucleosides in tRNA, which improves the aa-tRNA selection rate and optimizes the fitness of the tRNA in the P-site, is an important parameter that controls the reading frame maintenance.

3. Transfer RNA modification: influence on metabolism

3.1. tRNA modification as a regulatory device – a hypothesis

Different environmental changes have been shown to influence the degree of tRNA modification [7,24], which may result in inefficient or aberrant translation. Thus, the degree of tRNA modification may act as a regulatory device by influencing the translation of a certain mRNA as a response to a specific environmental change. However, a regulatory device must be specific, i.e. it should target a particular cellular reaction and the translational defect should primarily affect the translation of one mRNA compared to mRNAs in general. Several ways in which undermodified tRNA may implement the required specificity may be envisioned [7]. First, the deficiency of a specific modified nucleoside may not be the same in all tRNA species it is normally part of. Moreover, the function of a modified nucleoside, which is present in more than one tRNA species, is tRNA dependent [20]. Second, an element of specificity may be implemented by the influence exerted by the undermodified tRNA on the codon context sensitivity, on codon choice, or on frameshifting at certain sequences in the mRNA. Some sequences, such as the programmed frameshifting sites, which are present in both bacteria and eukaryotes, but also other sites in the mRNA are prone to frameshifting [25]. Modified nucleosides prevent frameshifting [22,23,26–28] and the level of tRNA modification may thereby have a profound influence on gene expression by its effect on reading frame maintenance. Third, some modified nucleosides can influence the aminoacylation of tRNA (reviewed in [23]). Therefore, the degree of modification may regulate the charging level of a subpopulation of tRNA that in turn will influence the efficiency of translation. Thus, there are several ways in which the degree of tRNA modification influences the efficiency and fidelity of translation, which may result in a specific effect on gene expression. Clearly tRNA modification may act as a regulatory device through various mechanisms but so far there are no good examples of how and why such links exist. For further discussion of this hypothesis see [7].

3.2. Lack of the hydroxyl group of ms^2io^6A37 prevents growth on citric acid cycle intermediates and changes iron metabolism

Synthesis of ms^2io^6A37 in *Salmonella typhimurium* occurs in several steps:

$$A37^{miaA} \rightarrow i^6A37^{miaB,C} \rightarrow ms^2i^6A37^{miaE} \rightarrow ms^2io^6A37$$

A *miaE* mutation results in ms^2i^6A37 instead of ms^2io^6A37

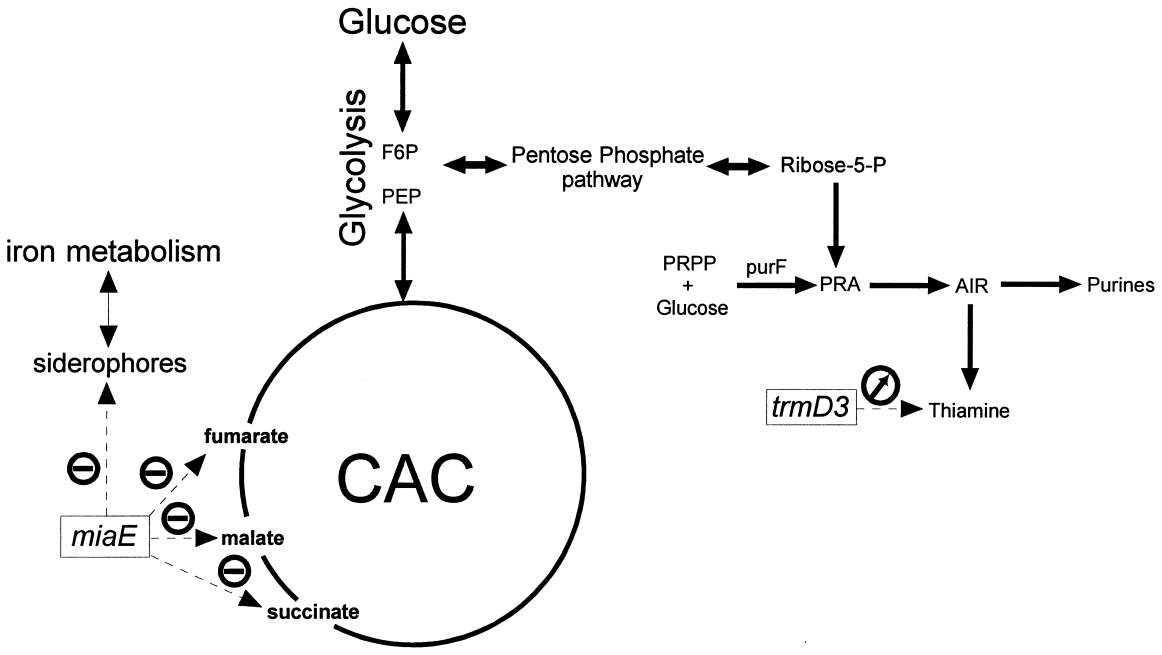

Fig. 2. Hypomodification of tRNA alters central or intermediary metabolism. The mutant (*miaE*) defective in the hydroxylation of ms^2i^6A37 to ms^2io^6A37 in *S. typhimurium* is unable (denoted −) to grow on succinate, malate, and fumarate and to excrete siderophores. The mutant (*trmD3*) deficient in m^1G37 apparently derepresses the level of thiamine (denoted ↗) making a *purF* mutant able to grow without thiamine.

in the tRNA. Such modification deficiency does not influence the growth rate of the cell or the activity of a nonsense suppressor tRNA. However, the *miaE* mutant is unable to grow on the citric acid cycle intermediates succinate, fumarate or malate [29]. This growth impairment is caused neither by a deficiency in the uptake of the carbon compounds, nor by a defective citric acid cycle enzyme or defects in the respiratory chain [30]. Thus, it seems that *S. typhimurium* senses the hydroxylation status of the isopentenyl side chain of the A adjacent to the anticodon [30]. Unlike the wild type strain, the *miaE* mutant is not excreting siderophores, suggesting that the iron metabolism may be impaired. Since iron is in higher demand in cells growing in succinate medium compared to glucose medium [31], the primary effect of the non-hydroxylated isopentenyl side chain may be on the iron metabolism. Indeed, addition of siderophores to the growth medium suppresses the growth defect in succinate medium. Suppressors have been isolated that suppress both the growth phenotype and the excretion of siderophores. These suppressor mutations reduce the expression of putative transcription factors indicating that the lack of the hydroxyl group of ms^2io^6A37 may influence a regulatory network operating in the central metabolism.

3.3. Deficiency of m^1G37 influences thiamine metabolism

The synthesis of thiamine and purines in *S. typhimurium* shares a common intermediate, 4-aminoimidazole ribotide (AIR) (Fig. 2). Therefore, a mutation in the gene (*purF*), which encodes the first enzyme in the purine pathway, blocks the synthesis of both purines and thiamine. Thus, a *purF* mutant requires both adenine and thiamine for growth when glucose is the carbon source. However, under certain physiological conditions such as anaerobic growth, or growth on non-glucose carbon sources, thiamine is not required, since the *a*lternative *p*yrimidine *b*iosynthetic (APB) pathway is activated [32]. The formation of m^1G37 is catalyzed by the *trmD* gene product, the tRNA(m^1G37)methyltransferase. Introduction of the *trmD3* mutation, which results in m^1G37 deficient tRNA and +1 frameshifts errors [26], into a *purF* mutant induces ability to grow if only adenine is supplemented to glucose medium. This thiamine independent growth of the double mutant *purF2085 trmD3* requires an active APB pathway and an active pentose phosphate shunt. Several other mutations (*miaA, miaE, sufA6*, and *sufB2*), which, like *trmD3*, also affect translation fidelity and induce slow growth, did not show thiamine independent growth in a *purF* background. Therefore, the *trmD3* induced thiamine independent growth phenotype seems to be specific to m^1G37 deficiency and not to a general translational defect or slow growth as such. According to our working hypothesis, aberrant translation of some key mRNA(s) is responsible for the changed regulatory response of thiamine synthesis.

3.4. tRNA modification influences the virulence of Shigella

The modified nucleoside Q34 is present in the wobble position (position 34) of a subset of tRNAs. A mutant (*tgt*) of *Escherichia coli* lacking Q34 in its tRNA grows as well as the wild type strain although the survival in stationary phase is impaired [33]. Lack of Q also increases read-through of UAG codons by $tRNA^{Tyr}$ [34–36] and influences codon choice [37]. On the other hand, the absence of ms^2i^6A37 in tRNA has a strong influence on the activity of tRNA and reduces the growth rate of the mutant by 20–50% depending on the growth medium [38]. Thus, whereas Q34 has only a minor effect on the growth and physiology of the bacterium, the presence of ms^2i^6A37 influences these parameters profoundly.

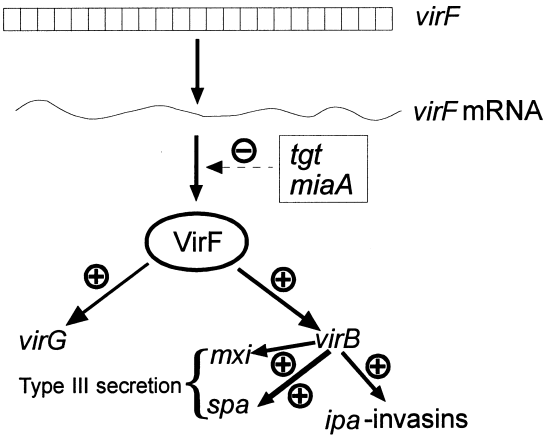

Fig. 3. tRNA modification is required for the virulence of *Shigella*. The VirF protein positively regulates *virG* and *virB* gene expression. The VirB protein is in turn a positive regulator of the *ipa*, *mxi*, and *spa* operons. Lack of Q34 or ms^2i^6A37 as in *tgt* and *miaA* mutants, respectively, reduces the level of VirF and thereby the expression of the virulence associated *ipa* genes. However, the level of *virF* mRNA is not affected by these mutations.

The virulence of *Shigella* is dependent on the activity of the regulator protein VirF, which belongs to the *araC* protein family. The VirF protein positively regulates the expression of the transcriptional activator VirG, which is required for the intracellular spreading of the bacterium, and another transcriptional activator, VirB, which in turn activates the expression of other virulence operons; the *ipa*, *mxi* and *spa* operons [39]. These operons encode the invasins IpaBCD and the Mxi-Spa type type III secretion apparatus (Fig. 3). Thus, VirF seems to be the key regulatory protein in the network that regulates the virulence of *Shigella*.

A *Shigella tgt* mutant is 50% less virulent than the wild type as judged by the expression of hemolytic activity, which is a measurement of the production of the Ipa proteins. This effect is correlated with a 50% reduction of the VirF protein although no effect on the level of *virF* mRNA was observed [40]. Similarly, ms^2i^6A37 deficiency, as in a *miaA* mutant, reduces the hemolytic activity to 10–20% and the level of VirG and VirF to about 10% of the level in the wild type. Still no effect on the *virF* mRNA level was noticed [41]. Specific overexpression of the *virF* mRNA restores the hemolytic activity in both mutants suggesting that the primary target for those two tRNA modifications is the translation of *virF* mRNA. This fact links the virulence of *Shigella* to the translation capacity of the bacterial cell, a feature that may be important in the ever-changing environment of a pathogenic bacterium.

4. Epilogue

Some modified nucleosides in tRNA are involved in maintaining the correct reading frame. A reduced level of such a modified nucleoside will therefore result in a reduced level of a protein. If this protein is a regulatory protein belonging to a regulatory network it will have strong influence on the expression of genes in this network. Perhaps mRNAs that encode regulatory proteins, which are normally expressed in low amounts in the cell, are especially sensitive to the tRNA modification level and thereby will be the target mRNAs that link metabolism to the translational capacity of the cell. Future work will show if this is a general feature and if this postulated regulatory device is important for the cell to adapt to a changing environment.

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