In this issue of the Biochemical Journal, Watanabe and colleagues disclose another fascinating facet of the mitochondrial protein synthesis machinery: one of the two nematode mitochondrial elongation factors Tu, EF-Tu1, specifically recognizes the D-arm of T-armless tRNAs via a 57-amino-acid C-terminal extension that compensates for the reduction in tRNA structure. This principle provides a paradigm for the evolutionary events thought to have ignited the transition from an ancient ‘RNA world’ to the ‘protein world’ of today.

Key words: deletion of D- and T-arm, elongation factor Tu, mitochondrion, nematode, RNA world, tRNA.

INTRODUCTION

In nematode mitochondria, not a single cloverleaf tRNA participates in protein synthesis. Instead, among the 22 tRNAs encoded in the organellar genome, 20 lack the T-arm module and two have a deletion of the D-arm. In this issue of the Biochemical Journal, Watanabe and colleagues [1] reveal the molecular basis of an idiosyncrasy of the mt (mitochondrial) protein synthesis machinery: EF-Tu1 (elongation factor Tu1), one of the two nematode mt elongation factors Tu encoded in the nuclear genome, specifically recognizes the D-arm of T-armless tRNAs via a 57-amino-acid C-terminal extension that compensates for this reduction in tRNA structure. The two D-armless tRNAs are recognized by the second EF-Tu protein, EF-Tu2, which has a C-terminal extension of approx. 16 amino acids relative to the bacterial counterparts. Both EF-Tu1 and Tu2 have lost their capacity to bind canonical cloverleaf tRNA ([2], see also [2a]) (Figure 1).

EVOLUTION OF EF-Tu

EF-Tu (Tu stands for thermo-unstable) forms the elongation factor subfamily of GTPases together with EF-G and IF2 (initiation factor 2). All three proteins have homologues in Bacteria, Archaea and Eukarya, suggesting that they arose by gene-duplication events that predate the divergence of the Kingdoms of life. This is particularly obvious for the two elongation factors. Evidence for the model that an ancient EF-Tu gave rise to its larger sibling EF-G [3] comes from the tandem arrangement of the two genes in bacteria and their similarity in sequence, structure and domain composition: the N-terminal part of EF-G resembles EF-Tu, whereas the C-terminal expansion of EF-G mimics the tRNA and is probably a more recent evolutionary acquisition. In principle, this scenario of early EF-Tu/EF-G evolution could be regarded as a precedent for the findings of Watanabe and co-workers [1]: also in nematode mitochondria, an EF-Tu progenitor after gene duplication diversified on the basis of a C-terminal extension.

FUNCTIONAL ROLE OF EF-Tu IN PROTEIN SYNTHESIS

EF-Tu, one of the most abundant cellular proteins, promotes the binding of aa-tRNA (aminoacyl-tRNA) to the A site (acceptor site) of the ribosome. The protein’s functional form is a ternary complex (aa-tRNA–EF-Tu–GTP), which also protects aa-tRNA from premature hydrolysis in the cellular milieu. Delivery of aa-tRNA to the A site is a process of at least two steps: in the first decoding step, aa-tRNA binds to the A site in a low-affinity state that allows discrimination of correct versus incorrect codon–anticodon interaction; in the second accommodation step, which is coupled to exit of deacylated tRNA from the E site, the aa-tRNA is released from the ternary complex and fully accommodated in the A site, while EF-Tu-GDP leaves the ribosome (reviewed in [4]). Uhlenbeck and co-workers [5] have demonstrated further that bacterial EF-Tu binds aa-tRNAs in a uniform manner. The thermodynamic contributions of the amino acid and the tRNA to the overall binding affinity are independent of each other and compensate for one another in correctly acylated tRNAs.

IDIOSYNCRASIES OF MITOCHONDRIAL PROTEIN SYNTHESIS

Owing to the bacterial origin of mitochondria, their protein synthesis machinery is more closely related to the bacterial one than to that of the eukaryotic hosts. This is exemplified by bacterial traits such as the use of formylated initiator Met-tRNAMet and a bacterial-like sensitivity to antibiotics. Governed by simplicity and economy, mt translation additionally displays a number of idiosyncrasies that distinguishes it further from eukaryotic cytoplasmic protein synthesis. Beyond the 22 tRNAs and two rRNAs, almost all mt genomes encode solely 12–14 polypeptides that are involved in oxidative phosphorylation; all other mt functions are encoded in the nucleus. Also, the distinct decoding mechanism most mitochondria utilize permits deciphering of all codons by a smaller number of tRNAs than is required to read the universal genetic code; thus, in most animal mitochondria, the 22 mitochondrially encoded tRNAs suffice to maintain organelle-specific protein synthesis.

Compared with bacterial and eukaryotic cytoplasmic protein synthesis machineries, there is a clear trend in mt protein synthesis to replace RNA structure with protein. Almost half of the rRNA contained in the bacterial ribosome is replaced with proteins in mammalian mt ribosomes. Interestingly, mt ribosomal proteins for which the binding sites on tRNA are reduced or lost carry N- or C-terminal extensions relative to their bacterial counterparts [6]. This type of structural compensation illustrates what is thought to

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have happened during the transition from an 'RNA world' to the extant 'protein world'; namely, that smaller peptides were expanded to larger polypeptides in order to take over RNA function.

A reduction of RNA structure is also seen in tRNA: in animal mitochondria, one serine-specific tRNA isoacceptor, reading AGY codons, carries a truncation of the D-arm. The second cloverleaf-like serine isoacceptor lacks the extended extra arm, a hallmark of canonical serine tRNAs, in addition to several other deviations from canonical tRNA structures [7]. The single mammalian mt SerRS (mt tRNA-synthetase) charging these two isoacceptors recognizes the T-loop of the truncated tRNAser, but additionally requires the interaction of the D- and T-loop for recognition of the cloverleaf-like tRNAser. Thus the mt SerRS has entirely given up the recognition mechanism utilized by pro- and eu-karyotic cytoplasmic SerRS enzymes, namely recognition of the long extra arm. The unique mode of tRNA binding by mt SerRS is accomplished via small N- and C-terminal extensions and a patch of basic amino acids, and these interaction sites are utilized differently for binding of the two seryl-tRNAs [7].

The present and previous work of Watanabe and colleagues ([1], and references therein) has revealed that EF-Tu1 specifically recognizes T-armless, and EF-Tu2 the D-armless tRNAs in nematode mitochondria. Since EF-Tu proteins recognize the acceptor arm and T-stem, a T-arm deletion is structurally more severe than a D-arm deletion. Although both EF-Tu1 and -Tu2 carry C-terminal extensions, their lengths differ, with 57 amino acids in Tu1 and 16 amino acids in Tu2 ([2], see also [2a]). This length difference may indeed reflect a higher level of complexity required to compensate for the absence of the T-arm versus D-arm. Interestingly, mammalian mt EF-Tu also carries a C-terminal extension (11 amino acids in bovine mt EF-Tu) proposed to be involved in tRNA recognition [8]. Assuming a single EF-Tu in mammalian mitochondria, this short extension may have sufficiently improved binding of their single D-armless tRNAser.

mt EF-Tu in Saccharomyces cerevisiae provides another example of gain of function. After the EF-Tu–GDP complex has left the ribosome, the protein depends on being recycled by the exchange factor EF-Ts, which assists in substituting GTP for the GDP. However, in S. cerevisiae and its close relatives, EF-Tu has become independent of its exchange factor and the gene for EF-Ts is lost from the genomes. Yet, unlike the situation with EF-Tu1 and -Tu2 in Caenorhabditis elegans, the changes in protein sequence required for such a gain of function are subtle, and no additional domain was recruited [9].

DRIVING FORCES OF MITOCHONDRIAL EVOLUTION

The finding that nematode mt genomes encode truncated tRNAs might be explained by evolutionary pressure to reduce genome size. However, most nematode mt genomes range from

Figure 1  Nematode EF-Tu1 and -Tu2, originating from a gene-duplication event, evolved novel and mutually exclusive substrate specificities and lost their affinity for canonical tRNAs
13 to 14 kb, very similar in size to mammalian mt genomes (16–17 kb), and several nematodes even have mt genomes of up to 20 kb. Based on these slight size differences, it remains arguable whether the pressure to reduce genome size has fuelled the general reduction of tRNA size in nematode mitochondria. Also, why was the architectural principle of the tRNA cloverleaf broken for only one tRNA (Ser AGY) in mitochondria of placental mammals? Hardly for the sake of genome size reduction alone, but more likely for one tRNA gene in a single mt genome was very likely to have been silent in the beginning, i.e. without a noticeable effect on cell vitality. Indeed, it was shown that mitochondria harbouring at least 10% wild-type mt genomes exerted a fully protective effect against the manifestation of the biochemical defects caused by the human MERRF (myoclonic epilepsy associated with ragged-red fibres) tRNA$^{\text{Ser}}$/$T$ mutation [11]. As a result of genetic drift combined with a replicative advantage of the mutant mt genome, the mt genome with the truncated tRNA$^{\text{Ser}}$ gene finally could have taken over the entire population of mt genomes in progeny cells. This process may have provided an expanded time window to co-evolve other mt components encoded by the nucleus in order to compensate for the defect. A scenario simpler than this mt action/nuclear reaction model is conceivable as well: the mammalian mt protein synthesis machinery, including mt EF-Tu and SerRS proteins, may have simply been able to cope with the shorter tRNA$^{\text{Ser}}$ D-arm truncation. The D-armless tRNA$^{\text{Ser}}$ seems to still function suboptimally in mt protein synthesis, as inferred from results obtained in a mammalian mt in vitro translation system. However, AGY codons recognized by this isoacceptor are minor serine codons in the human mt genome, which could explain the tolerance towards this isoacceptor [12]. Furthermore, eukaryotic cytoplasmic EF-1α, the pendant of bacterial EF-Tu, is usually longer than bacterial EF-Tu, typically approx. 17 amino acids [8]. Thus the mammalian mt EF-Tu, expected to be an orthologue of EF-1α, may have had a C-terminal extension from the beginning, which instantly increased binding affinity for the D-armless tRNA. A larger evolutionary leap was taken when nematode mitochondria usurped mt EF-Tu1 with a 57 nt extension, but again, an EF-Tu carrying a similar extension may have pre-existed in mitochondria of the nematode progenitor when the T-arm truncation of mt tRNAs was established.

The asexual evolution of mt genomes, which means absence of recombination and genome segregation typical of meiotic processes, is thought to have resulted in a gradual loss of fitness, which the eukaryotic cell has compensated for in order to maintain the function of this indispensable organelle [13]. In turn, mt evolution has probably been fuelled by access to the copious coding and recombination capacities of nuclear genomes and their effective mechanisms of protein evolution. Among the cornucopia of cases of nuclear–mitochondrial co-evolution is human mt-tRNA$^{\text{Ser}}$, which primarily populates a non-cloverleaf conformation in its unmodified state. To fix the problem, the nucleus has provided an A9-N1-methyltransferase that stabilizes the cloverleaf [14]. Another example comes from marsupials, a group of non-placental mammals: here, mt-tRNA$^{\text{Lys}}$ is corrected by a C-to-U editing event [13]. Nuclear–mitochondrial co-evolution has in some cases been pushed even further: in several protozoa, not a single tRNA is encoded in the mt genome and all mt tRNAs are imported from the nucleus [15]. An intermediate evolutionary state is found in marsupials, where cytosolic tRNA$^{\text{Lys}}$ is imported into mitochondria to compensate for the degeneration of the mt tRNA$^{\text{Ser}}$ gene to a non-functional pseudogene [16].

Allegorically speaking, mitochondria may, in a way, be viewed as the ‘spoiled and neglected adoptive children’ of the eukaryotic cell, taking for granted that their ‘rich parents’ (i.e. the nuclear genome) will ‘fix what they screw up’. In this sense, Watanabe and co-workers have played a central role in disclosing such lapses.

REFERENCES