

Is yeast on its way to evolving tRNA editing?

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In human mitochondria, genes for tRNA^{Tyr} and tRNA^{Cys} overlap by a single nucleotide. From polycistronic precursors, a 3'-truncated upstream tRNA^{Tyr} is released, missing the overlapping position. A subsequent editing reaction restores this position. Similar mitochondrial tRNA gene overlaps exist in all metazoans, but not in organisms such as yeast or *Escherichia coli*. Therefore, we asked whether tRNA overlaps are processed in these organisms. Corresponding constructs were introduced and transcripts tested for processing and editing in *E. coli* and yeast. *E. coli* produces only one functional tRNA from these precursors, indicating that tRNA overlaps are incompatible with its processing pathway. In contrast, yeast processes overlapping tRNAs similar to human mitochondria, releasing a 3'-truncated upstream tRNA. This tRNA is restored in an editing-like event, although yeast does not carry a corresponding endogenous editing substrate. These findings support the hypothesis of the evolution of editing by recruitment of a pre-existing and promiscuous editing enzyme.

Keywords: *Escherichia coli*; overlapping genes; RNA editing; *Saccharomyces cerevisiae*; tRNA processing

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INTRODUCTION

In mitochondrial genomes of metazoans, overlapping tRNA genes have been described (Yokobori & Pääbo, 1995a,b, 1997; Tomita *et al*, 1996; Reichert *et al*, 1998). Depending on the organism, the overlaps span a region of 1–6 nucleotides. Adjacent tRNAs are transcribed as part of a multicistronic precursor, and processing releases a complete downstream tRNA (carrying the overlapping nucleotides at its 5' end) and a 3'-truncated upstream tRNA that misses the overlapping region. Subsequently, this tRNA is restored by an unknown nucleotide-incorporating activity (Yokobori & Pääbo, 1995a,b; Reichert *et al*, 1998). In the human mitochondrial genome, the overlap between genes for tRNA^{Tyr} and tRNA^{Cys}

consists of a single A residue, representing the last nucleotide of tRNA^{Tyr} as well as the first nucleotide of tRNA^{Cys} (Reichert *et al*, 1998).

Here, we investigated whether overlapping tRNA genes can exist in organisms that naturally do not contain such overlaps and whether maturation pathways of these organisms lead to functional transcripts. The results indicate that the processing machinery of *Escherichia coli* is not compatible with overlapping tRNAs, whereas in yeast, processing as well as tRNA editing could be found in the cytosol/nucleus. These results support the evolutionary scenario of RNA editing, describing the acquisition of a pre-existing activity as an editing enzyme.

RESULTS AND DISCUSSION

E. coli and *Saccharomyces cerevisiae* (representing prokaryotes and eukaryotes) do not carry overlapping tRNA sequences in their genomes. This fact led to the question of whether these organisms are able to process overlapping tRNA precursors in a way similar to that of metazoans, which contain such gene overlaps in their mitochondrial DNA. Therefore, both organisms were tested for their ability to process artificially introduced overlapping tRNA precursors.

Processing of overlapping tRNA precursors in *E. coli*

The following constructs were cloned into plasmid pBAD30 without 5' extensions and introduced into *E. coli* CA244 (Fig 1A): in construct *sup-tRNA^{Tyr}-1/T5 tRNA^{Cys}*, two tRNA genes (*E. coli* suppressor tRNA^{Tyr}, phage T5 tRNA^{Cys}) were overlapping, as position A73 of the suppressor tRNA was not encoded, resembling the situation in human mitochondria. The second construct *sup-tRNA^{Tyr}+A/T5 tRNA^{Cys}*, where this position was encoded, served as a control. *sup-tRNA^{Tyr}* and T5 tRNA^{Cys} were chosen to distinguish introduced tRNAs from endogenous transcripts. All constructs were expressed by arabinose induction, and cells were grown on selective Luria-Bertani agar plates (30 µg/ml ampicillin). Subsequently, total RNA was prepared and the 3' end of *sup-tRNA^{Tyr}*—resulting from the overlapping construct—was determined by ligation of the RNA to an RNA/DNA oligonucleotide, followed by reverse transcription-PCR (RT-PCR), cloning and sequencing of individual clones (Fig 2). *sup-tRNA^{Tyr}* released from the nonoverlapping construct carried an A residue at position 73 in 100% of the cases. In contrast, the corresponding tRNA from the overlapping construct showed in 68% (23 out of 34 clones)

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a G residue at position 73 and a complete (65%, 22 clones) or partial (3%, one clone) CCA end, whereas a further 32% (11 out of 34) showed two G residues plus complete or partial CCA sequence. As both G residues at the 3' end of tRNA^{Tyr} probably result from the 5' part of tRNA^{Cys}, a truncated sup-tRNA^{Tyr} was obviously not released in *E. coli*.

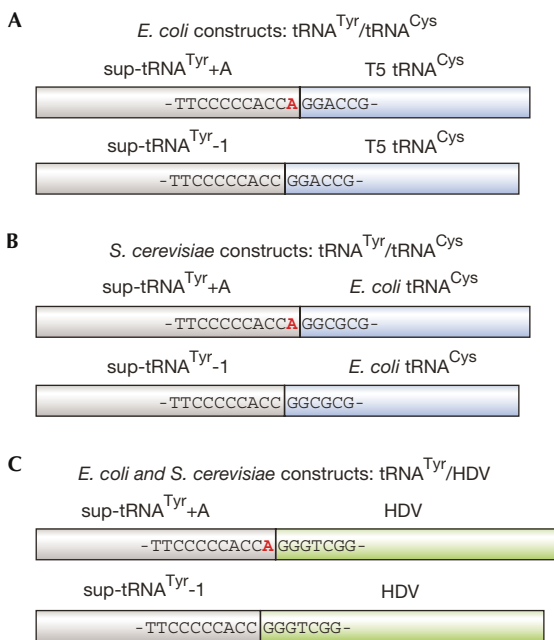


Fig 1 | Expression constructs. (A,B) Constructs resembling overlapping tRNA genes: sup-tRNA^{Tyr} is located upstream of tRNA^{Cys} from phage T5, for expression in *Escherichia coli* (A), and from *E. coli*, for expression in *Saccharomyces cerevisiae* (B), respectively. In sup-tRNA^{Tyr} + A/tRNA^{Cys}, sup-tRNA^{Tyr} ends with position 73 (A, red), whereas in sup-tRNA^{Tyr}-1/tRNA^{Cys}, this position is missing. (C) Constructs of sup-tRNA^{Tyr} linked to HDV ribozyme. In tRNA^{Tyr} + A/HDV, the tRNA carries position A₇₃ (red). In tRNA^{Tyr}-1/HDV, the tRNA sequence ends without this position.

In addition, we tried to detect the T5 tRNA^{Cys} located downstream. As standard procedures of tRNA circularization and RT-PCR for the simultaneous analysis of 5' and 3' ends of tRNA (Yokobori & Pääbo, 1995a) did not lead to any product, an alternative strategy was applied. An RNA/DNA-tagging oligonucleotide was ligated to the tRNA 3' end and the ligation product amplified by RT-PCR using two different primer sets (P1/P2 and P1/P3; Fig 3A). Complementary DNA amplification of an *in vitro*-transcribed T5 tRNA^{Cys} using these primers served as a positive control and resulted in products of 62 and 123 base pairs (bp), respectively (Fig 3B). In a further control PCR, sup-tRNA^{Tyr} was amplified as an *in vitro* transcript as well as one present in the tRNA preparation, indicating that the RNA preparation contained amplifiable material. The *in vitro* transcript resulted in a product of the expected size (66 bp; Fig 3B), whereas sup-tRNA^{Tyr} amplified from total RNA migrated at a higher position, as it carried position 73 plus the CCA end (70 bp; Fig 3B). In contrast, T5 tRNA^{Cys} from *E. coli* total tRNA preparation could not be amplified with any of the primer pairs, indicating that this tRNA is not expressed as a stable transcript in transformed *E. coli*.

In another strategy to express a 3'-truncated tRNA, constructs of sup-tRNA^{Tyr} lying immediately upstream of the sequence of human hepatitis delta virus ribozyme (HDV) were designed and expressed in *E. coli* (Fig 1C; Wu & Huang, 1992; Schürer et al, 2002). *In vitro*, such precursor transcripts were processed by the cleavage reaction of the ribozyme, releasing a suppressor tRNA^{Tyr} with (sup-tRNA^{Tyr} + A/HDV) or without position 73 (sup-tRNA^{Tyr}-1/HDV; not shown). Sequence analysis of 3' ends showed that sup-tRNA^{Tyr} released from the non-overlapping construct carried the encoded A₇₃ plus a complete or partial CCA terminus (not shown), whereas products resulting from the overlapping construct showed a G residue at position 73. Most of the clones (92%) represented a sup-tRNA^{Tyr} with G₇₃ and complete (67%, 8 out of 12 clones) or partial (25%, 3 out of 12) CCA end, whereas 8% (1 out of 12) showed two G residues plus a CCA terminus (Fig 2). Therefore, as in the tRNA^{Tyr}/tRNA^{Cys} constructs, none of the clones showed a sup-tRNA^{Tyr} in which position A₇₃ was restored. The G residues at the tRNA 3' end probably originate from the precursor transcript. As in the case of T5 tRNA^{Cys}, no HDV RNA

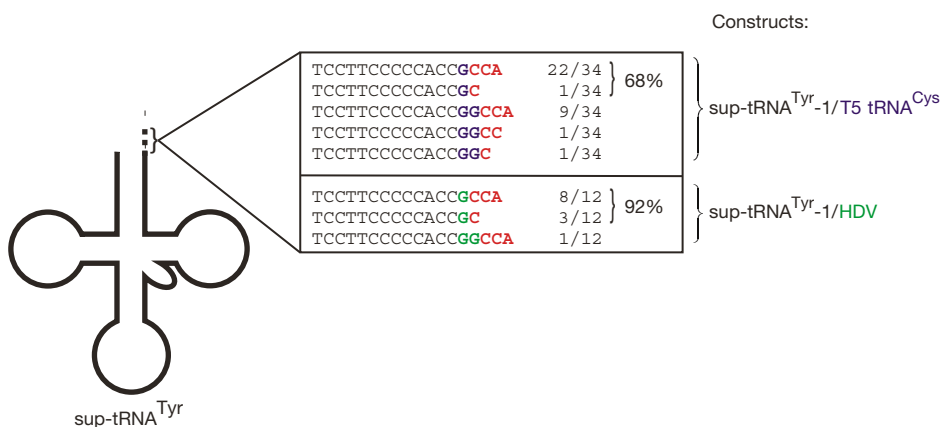


Fig 2 | Sequence analysis of upstream sup-tRNA^{Tyr} expressed in *Escherichia coli*. All molecules carry the diagnostic G residue at position 73 (blue, 5' of the CCA terminus, red) that represents the first position of the downstream RNA. This indicates that in both constructs (sup-tRNA^{Tyr}-1/T5 tRNA^{Cys}, sup-tRNA^{Tyr}-1/HDV), tRNA^{Tyr} is not released as a 3'-truncated tRNA, but as a complete molecule.

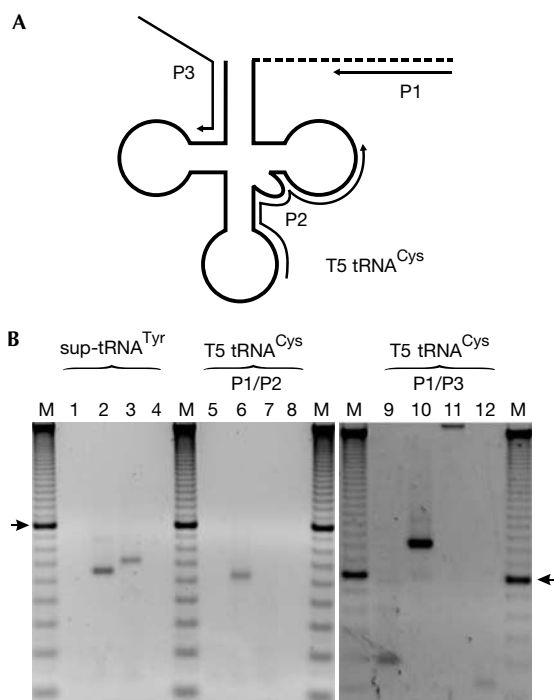


Fig 3 | Analysis of downstream T5 tRNA^{Cys} in *Escherichia coli*. (A) Schematic representation. After fusing the 3' end of the tRNA to the tag oligonucleotide (dashed line) and cDNA conversion (primer P1), several primer pairs were used for PCR amplification. (B) PCR products were separated on agarose gels. Left panel: lane 1, negative RT control without RNA; lane 2, sup-tRNA^{Tyr} *in vitro* transcript lacking A73 and CCA terminus (66 bp; positive control); lane 3, sup-tRNA^{Tyr} in *E. coli* RNA preparation (70 bp; positive control); lane 4, PCR blank control. Central and right panel: lanes 5,9, negative RT controls without RNA; lanes 6,10, *in vitro*-transcribed T5 tRNA^{Cys} (positive controls: 62 and 123 bp); lanes 7,11, *in vivo*-expressed T5 tRNA^{Cys} is not amplifiable; lanes 8,12, PCR blank controls. M, DNA size standard (10 bp ladder; 100 bp band is indicated by arrows).

was detected in RT-PCR analysis, indicating that the ribozyme was rapidly degraded in *E. coli*.

The fact that bacteria and mitochondria have several features in common (genome organization, gene expression, etc.) indicates that mitochondria are of prokaryotic origin (Scheffler, 1999). Therefore, it is possible that overlapping tRNA genes of mitochondria are also a feature common to prokaryotes. However, our results demonstrate that *E. coli* is not able to make two functional tRNAs from overlapping precursors. An explanation for this discrepancy is that *E. coli* uses a set of endo- and exonucleases for tRNA 3' end processing (Ray & Apirion, 1981b; Deutscher, 1984, 1990, 1995; Li & Deutscher, 1994, 1996): the initial step is a cleavage reaction in the 3' trailer (here, represented by tRNA^{Cys}) by RNase E or RNase III (Ray & Apirion, 1981a; Apirion & Miczak, 1993; Deutscher, 1995; Li & Deutscher, 2002). Subsequently, the mature 5' end is produced by RNase P and the 3' end further trimmed by exonucleases. Obviously, cleavage of downstream tRNA^{Cys} by RNase P, leading to the release of a 3'-truncated upstream tRNA^{Tyr} (as observed *in vitro*, not shown), cannot

compete with this 3'-trailer trimming system. This situation can be explained by two scenarios. First, RNase P can be inhibited by long, unprocessed 3' ends (Altman *et al*, 1987). The 3' trailer of the downstream tRNA^{Cys} resulting from the expression vector may represent such an extension that interferes with RNase P activity. Second, transcription and processing of tRNA precursors are not independent in *E. coli*, but are linked and occur in parallel. Therefore, a nascent overlapping precursor transcript seems to be processed by RNase E as soon as the first tRNA has left the polymerase, before synthesis of the second tRNA is completed. This would initiate processing of the upstream tRNA by exo- and endonucleolytic 3' trimming, leading to degradation of downstream RNA. Alternatively, *E. coli* tRNAse Z might cleave downstream of the overlapping nucleotide, at the 3' end of tRNA^{Tyr}, leading to the release of a 5'-truncated tRNA^{Cys} that is then rapidly degraded by the RNA quality control system (Li *et al*, 2002). Such scenarios are strongly supported by the fact that neither T5 tRNA^{Cys} nor HDV ribozyme could be detected (Fig 3). Further support comes from the observation that upstream sup-tRNA^{Tyr} carries a G residue at position 73, which is probably derived from the RNA located downstream.

Processing overlapping tRNA precursors in *S. cerevisiae*

To analyse the processing of overlapping tRNA precursors in yeast, constructs consisting of *E. coli* sup-tRNA^{Tyr} and tRNA^{Cys} (sup-tRNA^{Tyr} + A/tRNA^{Cys}, sup-tRNA^{Tyr-1}/tRNA^{Cys}) were cloned into vector Y352_gal1 (Fig 1B). Constructs were expressed in *S. cerevisiae* BY 4741 growing in uracil-dropout medium containing galactose. Again, total RNA was isolated and tRNA 3' ends were analysed using the above-mentioned oligo-tagging procedure followed by cloning and sequencing of individual clones (Fig 4). As in *E. coli*, only processed tRNA molecules could be detected, but not precursor molecules, indicating that in both systems, cleavage reactions were comparably efficient, even at increased levels of induction (not shown). In yeast, however, only 2.3% of the clones (1 out of 44) carried the encoded overlapping G residue at the 3' end (plus two further nucleotides of the downstream tRNA^{Cys}), whereas 97.7% carried non-encoded C or A residues at these positions, suggesting that a truncated upstream tRNA^{Tyr} is released. Of the clones, 68% (30 out of 44) showed a C residue at the editing position plus a complete or partial CCA end, representing immediate CCA addition to the truncated 3' end of the tRNA. Interestingly, 25% (11 out of 44) carried an A residue at the editing position 73. As this A is not encoded, it must have been added after processing of the precursor. To examine the 5' end of downstream tRNA^{Cys}, the same RNA preparation used for tRNA^{Tyr} characterization was analysed by tRNA circularization and RT-PCR (Yokobori & Pääbo, 1995a). All clones (78 out of 78) showed a complete 5' end of tRNA^{Cys}, carrying the overlapping G residue at position 1 (Fig 4). This indicates that, in yeast, the precursor molecule is processed by an endonucleolytic cleavage 5' of tRNA^{Cys}, releasing a complete tRNA^{Cys} and a 3'-terminally truncated tRNA^{Tyr}.

As in *E. coli*, a second set of tRNA constructs (tRNA^{Tyr} fused to HDV ribozyme in overlapping and non-overlapping arrangements; Fig 1C) was introduced into *S. cerevisiae*. Here, it turned out that 79% (30 out of 38) of tRNA^{Tyr} clones had a restored A residue at the editing position 73 plus a complete (25 out of 38) or partial (5 out of 38) CCA end, whereas 5.4% carried a C residue at

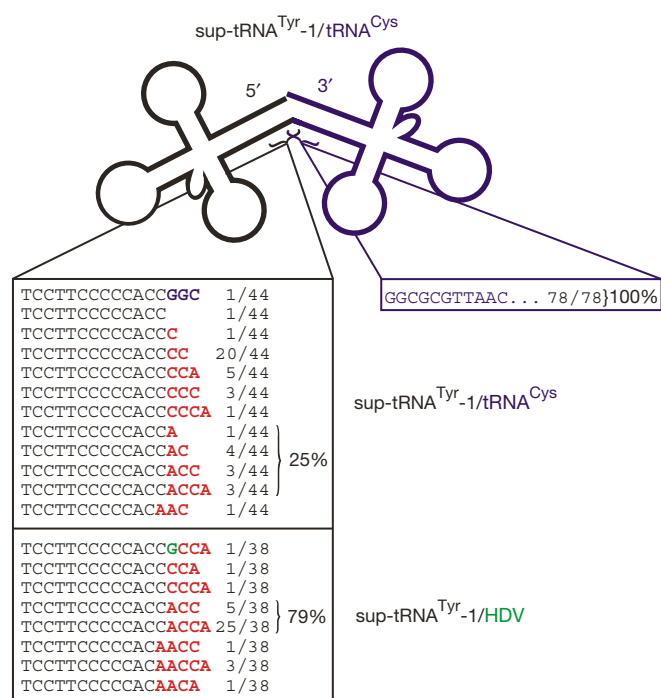


Fig 4 | Sequence analysis of 3' and 5' ends of tRNAs introduced into *Saccharomyces cerevisiae*. Whereas tRNA^{Cys} (blue) located downstream carried a complete 5' terminus including the overlapping first position (diagnostic G residue), upstream sup-tRNA^{Tyr} was released as a 3'-truncated molecule. An unidentified base-incorporating activity restored in 25%–79% of the cases the correct A residue at position 73 (bold character, red). This indicates that both tRNAs are released as stable transcripts and that yeast can catalyse editing-like reactions at the tRNA 3' end.

position 73. In all, 13% (4 out of 38) were obviously further truncated (positions 72 and 73) and were restored by the incorporation of two A residues with (partial) CCA addition. One single clone (2.6%) carried a G residue at position 73, which probably represents the overlapping first nucleotide of the HDV ribozyme.

This evidence indicates that yeast releases a truncated tRNA^{Tyr} in the processing of overlapping precursors and restores at least part of this pool of truncated tRNA by an editing-like activity, adding an A residue at position 73. These results lead to the following conclusions: in contrast to *E. coli*, the yeast tRNA maturation system is able to process overlapping tRNA precursors in a way similar to the human mitochondrial pathway. An endonuclease recognizes the bicistronic precursor and releases a complete downstream RNA (whether HDV ribozyme is released by the same mechanism or by a self-cleaving reaction was not determined). Possible candidates for such a reaction are RNase P (5'-end processing) and tRNase Z (3'-end processing). Whereas the typical RNase P cleavage reaction at the 5' end of tRNA^{Cys} would generate a 3'-truncated tRNA^{Tyr}, it is also possible that tRNase Z cleaves at a site shifted one position upstream relative to the standard site. A similar shift to the opposite direction was observed with pig tRNase Z, where the composition of the tRNA acceptor stem affects the site of trailer cleavage (Nashimoto et al, 1999). It

is not clear yet which of these enzymes is responsible for the release of a truncated upstream tRNA in yeast.

A possible candidate for the A inserting activity is poly(A) polymerase that might add several A residues to the 3' end of the tRNA. The surplus A residues might be removed by tRNase Z, leading to the release of a tRNA ending with A73 as a substrate for CCA addition. Indeed, some clones show a further truncated tRNA that was completed by the incorporation of two A residues at positions 72 and 73 followed by a complete or partial CCA end (Fig 4). But, although polyadenylated tRNAs were probable reaction intermediates in chicken mitochondria, where poly(A) polymerase is discussed as the editing activity (Yokobori & Pääbo, 1997), our analysis showed no such molecules. Another candidate is the CCA enzyme that generates the invariant CCA triplet at the 3' end of tRNAs. However, the recombinant yeast enzyme was not able to restore the missing A residue at position 73, but simply added CCA to the truncated tRNA substrate (not shown). Therefore, both poly(A) polymerase and CCA enzyme can probably be ruled out in editing activities in yeast, indicating that a different, as yet unidentified, enzyme is involved.

The question arises as to whether the edited sup-tRNA^{Tyr} is indeed functional and can be aminoacylated with tyrosine. As the anticodon is not a principal identity element in tRNA^{Tyr}, the suppressor anticodon does not affect the identity of the tRNA (Andoh & Ozeki, 1968). However, the editing position A73 represents a true tyrosine determinant (Fechter et al, 2000). As the base-incorporating activity restores exactly the nature of this position, the tRNA receives an important identity element by the editing event, thereby defining its tyrosine identity. However, as this transcript represents a prokaryotic tRNA, it lacks a typical eukaryotic identity element (base pair C1–G72). Introducing that element into *E. coli* tRNA^{Tyr}, Fechter et al (2000) showed that this tRNA is then readily accepted by the corresponding synthetase and is therefore a functional tRNA in yeast. Thus, an edited *E. coli* tRNA^{Tyr} is a functional molecule and needs just a slight adaptation (which does not affect the editing position A73) to be tyrosylated efficiently in the yeast system.

Evolutionary considerations

These results document that production of two tRNAs from an overlapping precursor and formation of truncated tRNAs are incompatible with the prokaryotic tRNA processing pathway. The mitochondrion, as a former prokaryote, obviously replaced this tRNA 3'-end maturation using trimming exonucleases with the endonucleolytic system of the host cell, in which specific endonucleases cleave close to, or at, the tRNA 3' end (Schürer et al, 2001). Because such an endonuclease activity is compatible with overlapping tRNA transcripts, the adaptation of the host maturation pathway was a prerequisite for the evolution of overlapping tRNA genes in mitochondrial genomes. Furthermore, the current results indicate that the former prokaryotic endosymbiont transferred not only essential primordial functions, but also most of its essential endogenous genes to its host, and relies now on the re-import of the corresponding proteins. It also adopted host functions, such as RNA maturation modes, that are now identified as typical mitochondrial RNA-processing reactions.

The fact that *S. cerevisiae* is able to edit a truncated tRNA is surprising, as this organism does not carry overlapping tRNA genes in its genome. Obviously, an editing activity is present in

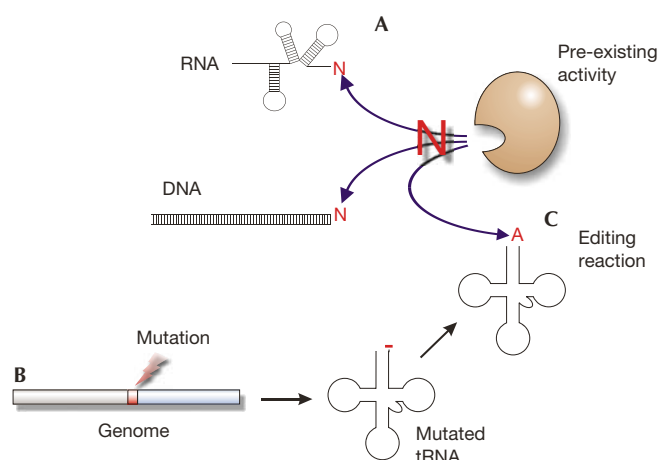


Fig 5 | Model for the evolution of RNA editing. (A) A nucleotide-incorporating enzyme is present as a pre-existing editing activity in the cell. (B) A mutation in a gene leads to the transcription of a (t)RNA molecule that has to be edited to regain its function. (C) The pre-existing activity is promiscuous and accepts the inactive transcript as a new substrate and restores its function. Thereby, an RNA-editing event is established.

yeast without having a corresponding editing substrate. Although this happens in the nucleus/cytosol and not in the mitochondria of yeast, this situation exactly represents the general scenario that is discussed for the evolution of RNA editing (Fig 5; Covello & Gray, 1993; Cavalier-Smith, 1997; Simpson *et al*, 2000). A prerequisite for the emergence of editing is a pre-existing enzymatic activity that catalyses a related reaction on some (RNA) substrates in the cell. This activity is promiscuous and accepts other (RNA) molecules as substrates. Then, a mutation—a deletion in the case of overlapping tRNA genes—occurs in a certain gene, leading to the expression of a nonfunctional transcript (here, 3'-truncated tRNA). Usually, such a mutation is deleterious, as the resulting product has lost its function. Here, however, the detrimental effect is neutralized by the promiscuous activity that restores the functional transcript. Consequently, the activity has now acquired an additional function as an editing enzyme. As this editing reaction removes the selective constraint from the mutated substrate gene, the editing event can be permanently established. Alternatively, it is also conceivable that yeast originally encoded editing substrates (overlapping tRNAs) and has lost them by mutation (base insertions). Nevertheless, even in that case, it is possible that a back mutation again creates overlapping tRNA molecules, (re)establishing an editing event in yeast.

As such a situation obviously exists in yeast, it seems that this organism might be on its way to acquiring a nuclear tRNA editing event similar to that observed in metazoan mitochondria. Further experiments will help to identify the nature of this possible candidate for a future editing enzyme.

METHODS

Preparation of DNA and RNA from *E. coli* and *S. cerevisiae*. Total DNA or RNA was prepared as described previously (Sambrook *et al*, 1989).

Construction of overlapping tRNA genes. See the supplementary information online.

Sequence analysis of tRNA 3' termini. tRNA was ligated to a DNA oligonucleotide (Purimex, Göttingen, Germany) carrying an RNA nucleotide at the phosphorylated 5' end (tag oligo pU-ATACTCATGGTCATAGCTGTT; Betat *et al*, 2004). cDNA synthesis was carried out by annealing primer P1 (AACAGCTAT GACCATG) to the tag oligonucleotide and using MMLV Reverse Transcriptase (New England Biolabs, Beverly, MA, USA) according to the manufacturer's instructions. cDNA was amplified in a standard PCR reaction containing 2% dimethyl sulphoxide using primer P1 and a primer specific for *E. coli* sup-tRNA^{Tyr}, *E. coli* tRNA^{Cys} or T5 tRNA^{Cys}. PCR products were cloned using a TA cloning kit (Invitrogen, Carlsbad, CA, USA). DNA sequencing was performed on an ABI Prism 3700 automated sequencer (Amersham Pharmacia Biotech, Freiburg, Germany).

Circularization of tRNA. The total RNA (4 μg) prepared was circularized as described previously (Yokobori & Pääbo, 1995a). cDNA was synthesized and amplified as described above, using primers specific for *E. coli* sup-tRNA^{Tyr} and *E. coli* tRNA^{Cys}.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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REFERENCES

- Altman S, Baer M, Gold H, Guerrier-Takada C, Lawrence N, Lumelsky N, Vioque A (1987) Cleavage of RNA by RNase P. In *Molecular Biology of RNA*, Inouye M, Dudock BS (eds) pp 3–15. Orlando, FL, USA: Academic
- Andoh T, Ozeki H (1968) Suppressor gene Su3+ of *E. coli*, a structural gene for tyrosine tRNA. *Proc Natl Acad Sci USA* **59**: 792–799
- Apirion D, Miczak A (1993) RNA processing in prokaryotic cells. *Bioessays* **15**: 113–120
- Betat H, Rammelt C, Martin G, Mörl M (2004) Exchange of regions between bacterial poly(A) polymerase and CCA adding enzyme generates altered specificities. *Mol Cell* **15**: 389–398
- Cavalier-Smith T (1997) Cell and genome coevolution: facultative anaerobiosis, glycosomes and kinetoplastan RNA editing. *Trends Genet* **13**: 6–9
- Covello PS, Gray MW (1993) On the evolution of RNA editing. *Trends Genet* **9**: 265–268
- Deutscher M (1990) Ribonucleases, tRNA nucleotidyltransferase, and the 3' processing of tRNA. *Progress Nucleic Acids Res Mol Biol* **39**: 209–240
- Deutscher MP (1984) Processing of tRNA in prokaryotes and eukaryotes. *CRC Crit Rev Biochem* **17**: 45–71
- Deutscher MP (1995) tRNA processing nucleases. In *tRNA: Structure, Biosynthesis and Function*, Söll D, RajBhandary UL (eds) pp 51–65. Washington, DC, USA: ASM
- Fechter P, Rudinger-Thirion J, Théobald-Dietrich A, Giegé R (2000) Identity of tRNA for yeast tyrosyl-tRNA synthetase: tyrosylation is more sensitive to identity nucleotides than to structural features. *Biochemistry* **39**: 1725–1733
- Li Z, Deutscher MP (1994) The role of individual exoribonucleases in processing at the 3' end of *Escherichia coli* tRNA precursors. *J Biol Chem* **269**: 6064–6071
- Li Z, Deutscher M (1996) Maturation pathway for *E. coli* tRNA precursors: a random multienzyme process *in vivo*. *Cell* **86**: 503–512
- Li Z, Deutscher MP (2002) RNase E plays an essential role in the maturation of *Escherichia coli* tRNA precursors. *RNA* **8**: 97–109
- Li Z, Reimers S, Pandit S, Deutscher MP (2002) RNA quality control: degradation of defective transfer RNA. *EMBO J* **21**: 1132–1138

- Nashimoto M, Tamura M, Kaspar RL (1999) Selection of cleavage site by mammalian tRNA 3' processing endoribonuclease. *J Mol Biol* **287**: 727–740
- Ray BK, Apirion D (1981a) RNAase P is dependent on RNAase E action in processing monomeric RNA precursors that accumulate in an RNAase E– mutant of *Escherichia coli*. *J Mol Biol* **149**: 599–617
- Ray BK, Apirion D (1981b) Transfer RNA precursors are accumulated in *Escherichia coli* in the absence of RNase E. *Eur J Biochem* **114**: 517–524
- Reichert A, Rothbauer U, Mörl M (1998) Processing and editing of overlapping tRNAs in human mitochondria. *J Biol Chem* **273**: 31977–31984
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour, NY, USA: Cold Spring Harbour Laboratory Press
- Scheffler IE (1999) *Mitochondria*. New York, USA: Wiley-Liss
- Schürer H, Schiffer S, Marchfelder A, Mörl M (2001) This is the end: processing, editing and repair at the tRNA 3'-terminus. *Biol Chem* **382**: 1147–1156
- Schürer H, Lang K, Schuster J, Mörl M (2002) A universal method to produce *in vitro* transcripts with homogeneous 3' ends. *Nucleic Acids Res* **30**: e56
- Simpson L, Thiemann OH, Savill NJ, Alfonso JD, Maslov DA (2000) Evolution of RNA editing in trypanosome mitochondria. *Proc Natl Acad Sci USA* **97**: 6986–6993
- Tomita K, Ueda T, Watanabe K (1996) RNA editing in the acceptor stem of squid mitochondrial tRNA(Tyr). *Nucleic Acids Res* **24**: 4987–4991
- Wu HN, Huang ZS (1992) Mutagenesis analysis of the self-cleavage domain of hepatitis delta virus antigenomic RNA. *Nucleic Acids Res* **20**: 5937–5941
- Yokobori S, Pääbo S (1995a) Transfer RNA editing in land snail mitochondria. *Proc Natl Acad Sci USA* **92**: 10432–10435
- Yokobori S, Pääbo S (1995b) tRNA editing in metazoans. *Nature* **377**: 490
- Yokobori S, Pääbo S (1997) Polyadenylation creates the discriminator nucleotide of chicken mitochondrial tRNA^{Tyr}. *J Mol Biol* **265**: 95–99