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Mutation of a highly conserved base in the yeast mitochondrial 21S rRNA restricts ribosomal frameshifting

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Abstract A mutation shown to cause resistance to chloramphenicol in *Saccharomyces cerevisiae* was mapped to the central loop in domain V of the yeast mitochondrial 21S rRNA. The mutant 21S rRNA has a base pair exchange from U_{2677} (corresponding to U_{2504} in *Escherichia coli*) to C_{2677}, which significantly reduces rightward frameshifting at a UU UUU UCC A site in a + 1 U mutant. There is evidence to suggest that this reduction also applies to leftward frameshifting at the same site in a − 1 U mutant. The mutation did not increase the rate of misreading of a number of mitochondrial missense, nonsense or frameshift (of both signs) mutations, and did not adversely affect the synthesis of wild-type mitochondrial gene products. It is suggested here that ribosomes bearing either the C_{2677} mutation or its wild-type allele may behave identically during normal decoding and only differ at sites where a ribosomal stall, by permitting non-standard decoding, differentially affects the normal interaction of tRNAs with the chloramphenicol resistant domain V. Chloramphenicol-resistant mutations mapping at two other sites in domain V are described. These mutations had no effect on frameshifting.

Key words Chloramphenicol resistance · frameshifting Mitochondria · 21S rRNA · Yeast

Introduction

Wild-type ribosomes may shift the reading frame (+ 1, − 1), while translating an mRNA sequence, and this phenomenon is obligatory for the synthesis of particular proteins (reviewed by Atkins et al. 1990; Hatfield et al. 1992). Two systems of frameshifting to nearby bases appear to operate. In one, frameshifting is governed by specific shift sites (see Brierley et al. 1992 and references therein) and secondary structure configurations in the mRNA sequence (Brierley et al. 1989; Chamorro et al. 1992; Gesteland et al. 1992; Parkin et al. 1992; ten Dam et al. 1990), the latter serving (through structural perturbations of the ribosome?) as brakes, to impede ribosomal progression (Tu et al., 1992) and ultimately precipitating tRNA uncoupling and recoupling at the shift site (reviewed in Atkins and Gesteland 1994). In the second system ribosomal frameshifting is precipitated merely by low tRNA availability at the shift site (Kawakami et al. 1993), without a prior requirement for tRNA uncoupling (Farabaugh et al. 1993). In a third system (Huang et al. 1988; Le et al. 1993) frameshifting is achieved by ribosomal hopping over longer distances. Excluding some complicating aspects of hopping (Weiss et al. 1990), it is clear that attempts to modulate the rate of frameshifting *at the ribosomal level* involve either seeking to change the postulated perturbation centre (if any) or manipulating the flexibility of the ribosome at the shift site, i.e. interfering with its in-built capacity for inaccurate decoding. In this paper we describe a mutation in the 21S mitochondrial rRNA of yeast, which alters the ribosome's tolerance of inaccurate decoding. The observation that diploid cell growth and levels of wild-type mitochondrial gene products are normal in the strain suggests that the mutant rRNA may function in the same way as the wild-type in orthodox decoding, but result in greater observed accuracy than in the wild-type when anomalous decoding is facilitated.

A substantial body of evidence reveals that mutations in ribosomal proteins (Kirsebom and Isaksson 1985; Allen and Noller 1989; reviewed by Parker 1989), as well as alterations in ribosomal RNA, may have profound repercussions for fidelity of translation, even

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            250
             |
WT      ... ILE TRP THR ILE PHE PRO ALA VAL ILE LEU LEU ILE ILE ALA PHE ...
        ... ATT TGA ACA ATT TTT CCA GCT GTA ATT TTA TTA ATT ATT GCT TTC ...

                              +1

M5631   ... ILE TRP THR ILE PHE SER SER CYS ASN PHE ILE ASN TYR CYS PHE ...
        ... ATT TGA ACA ATT TTT TCC AGC TGT AAT TTT ATT AAT TAT TGC TTT ...

                              -1

M5701   ... ILE TRP THR ILE PHE GLN THR ...
        ... ATT TGA ACA ATT TTC CAG CTG TAA TTTTATTAATTATTGCTT ...
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Fig. 1 Sequence of the M5631 frameshift mutation, the M5701 frameshift mutation and the corresponding portion of the wild-type *COXII* gene. Translation leads to a TAA stop (in DNA) in both mutants, generating protein fragments of molecular weight 12,500 and 9,600 for M5631 and M5701, respectively, compared to the full-sized subunit II of cytochrome *c* oxidase of M_r 33,500. A region which can potentially form a weak secondary structure is *underlined*. The wild-type sequence of the *COXII* gene is from Fox (1979). Numbering of bases starts at the first potential initiation codon according to de Zamorczy and Bernardi (1986)

when the alteration is minor as in one striking example reported by Prescott and Dahlberg, (1990). These mutations often simultaneously affect frameshifting, missense and terminator read-through in the direction of increased accuracy (Melançon et al. 1992) or decreased fidelity (O'Connor and Dahlberg, 1993). Instances of altered accuracy that have been documented for both the 16S rRNA and 23S RNA include: alterations at positions G_{517} (O'Connor et al. 1992), C_{726} (Prescott and Dahlberg, 1990), C_{1469} (Allen and Noller 1991) in 16S rRNA and at U_{2555} (O'Connor and Dahlberg 1993) in 23S rRNA, all of which severely reduce accuracy. On the other hand, increased accuracy is observed with mutations at G_{2583} (Saarma and Remme 1982) and G_{2661} (Melançon et al. 1992) of *Escherichia coli* 23S rRNA, and when C is altered to G in the yeast mitochondrial 15S rRNA at the position equivalent to C_{1409} in the *E. coli* 16S rRNA (Weiss-Brummer and Hüttenhofer 1989).

The antibiotic chloramphenicol interacts with the central loop of domain V of the 23S rRNA (Moazed and Noller 1987) and interferes with peptide bond formation (Monro and Vazquez 1967; Celma et al. 1970; Ballesta and Lazaro 1990), possibly by preventing tRNA 3′ ends from being correcty juxtaposed at the A and P sites. Mutants that are resistant to this antibiotic appear exclusively to alter the peptidyltransferase center of 23S rRNA (see Vester and Garret 1988) and we reasoned that in so doing such mutations might also alter the proper interaction of tRNAs at the A- and P-sites and result in fidelity problems. We therefore investigated the effect of this type of mutation on frameshifting and here describe one chloramphenicol-resistant mutant which, in specific diploids, significantly reduces frameshifting rates in a *COXII* reporter gene, without having any apparent effect on the general level of translation.

The *COXII* gene codes for subunit II of cytochrome *c* oxidase. A + 1 T addition (Fig. 1) to the gene results in high level (20%) frameshifting (Weiss-Brummer and Hüttenhofer 1989). This permits 'leaky' growth of the mutant (Fox and Weiss-Brummer 1980). A -1T mutation at the same site also leads, to a lesser extent, to frameshifting and leaky mutant growth. In contrast, two + 1 T mutations at T-rich sequences are not leaky (Fig. 5). This mutant collection allows frameshifting modulation (whether up or down) to be tested in both left and right directions. In addition, the mitochondrial DNA codes for a single copy of each rRNA subunit, and specifies a limited number of structural proteins. These properties make the yeast mitochondrial system eminently suitable for studies of mutant ribosomal RNA.

Materials and methods

Strains and media

Genotypes and origins of yeast strains are given in Table 1. Yeast media were as follows. YD: 1% yeast extract, 3% glucose; YG: 1% yeast extract, 3% glycerol; YG + chloramphenicol: 1% yeast extract, 3% glycerol, 50 mM phosphate buffer, pH 6.2, 3.0 mg/ml chloramphenicol (Serva); WO: 1.7% Difco yeast nitrogen base, 5.2% ammonium sulfate, 3% glucose. Solid media contained, in addition, 2% agar.

Isolation of mitochondrial mutants conferring resistance to chloramphenicol

Mitochondrial mutations conferring resistance to antibiotics can only be isolated under conditions of obligatory mitochondrial translation. This condition is generated when a respiratory-competent cell is required to grow on a non-fermentable carbon source such as glycerol. The strain under investigation, 777-3A-M5631 containing a mutant reporter gene *COXII* (cytochrome c oxidase subunit II), exhibits leaky rather than no growth on glycerol at 30 °C and 36 °C. No growth is observed at 23°C (Table 3). In addition, 777-3A-M5631 and its parental strain 777-3A (*COXII*$^+$) both carry the recessive nuclear *op1* mutation (pet$^-$) and therefore

Table 1 strains of *Saccharomyces cerevisiae* used

Strain	Genotype		Remarks	References[a]
	Nuclear	Mitochondrial		
777-3A	α *ade1*, *op1*	*rho*$^+$, *mit*$^+$		1
777-3A-M5631	α *ade1*, *op1*	*rho*$^+$, *mit*$^-$	Derived from 777-3A	2
KL14-4A	**a**, *his1*, *trp2*	*rho*$^+$, *mit*$^-$, P^R_{454}, oli^r, C^R_{321}		3
KL-0	**a**, *his1*, *trp2*	*rho*^{0}		4
KL14-777-3A	**a**, *his1*, *trp2*	*rho*$^+$, *mit*$^+$	Cytoductant	5
KL14-777-3A-[C1-C5]	**a**, *his1*, *trp2*	*rho*$^+$, *mit*$^+$	C^R mutants derived from KL14-777-3A	This work

[a] 1, Kotylak and Slonimski (1977); 2, Weiss-Brummer et al. (1979); 3, Wolf et al. (1973); 4, Sakai et al. (1991); 5, H. Sakai, unpublished

Abbreviations: rho$^+$ mit$^+$, wild-type; rho$^+$ mit$^-$, point mutation in one of the mitochondrial structural genes; rho°, mitochondrial mutant with total loss of the mitochondrial DNA. All these mutants are respiratory-deficient when grown on a medium containing a non-fermentable carbon source such as glycerol

chloramphenicol-resistant mutations could not be directly isolated in either strain. Instead mitochondria of the strain 777-3A were transferred via cytoduction to the rho° strain KL-0. KL-0 possesses the wild-type nuclear allele for respiratory function (PET$^+$) and has the complementary mating type to 777-3A-M5631. The resulting new strain was named KL14-777-3A (Sakai and Weiss-Brummer, unpublished). Cells of the strain KL14-777-3A (10^6) were plated on selective media containing the antibiotic. After 1–2 weeks, spontaneous mutant colonies were isolated.

In 20 cases, the mutations were localized by the transfer of mitochondria to the rho° strain KL-0 via double kar crosses. Five strains exhibited resistance after the transfer, indicative of a mitochondrial location for the corresponding mutations. The strains were designated KL14-777-3A-[C1-C5]. Dujon and Jaquier (1983) had previously documented three mitochondrial chloramphenicol-resistant mutants. However none of these were available in a background that was isogenic in nuclear and mitochondrial content to either the reporter strain 777-3A-M5631, containing the + 1 frameshift mutation M5631 in *COXII*, or to other *COXII* and *COB* mutant strains. Only isogenic backgrounds guarantee the absence of artefacts arising from strain-specific DNA differences and on this account only newly isolated mutants were used in the present study.

Crosses

The crosses were performed at 30° C on WO medium. After segregation of the mitochondrial genomes, single colonies arising at 30° C on WO were replica plated to YG and incubated for about 8 days at 23°, 30°, 36° C. The presence of the antibiotic resistance markers cannot be tested directly because colonies with 'leaky' and 'reduced leaky' phenotypes do not grow very well on selective plates containing a non-fermentable carbon source. For this reason revertants (M5631 to wild-type) were isolated. These cells could then be tested on YG and YG plus chloramphenicol plates.

Cytoduction

For 'mitochondrial backcrosses' the mitochondrial genomes were transferred to the strain KL-O via cytoduction with the kar strain MS20/A1, as described by Lancashire and Mattoon (1979).

Growth rate determination

Spontaneous respiratory-competent (RC) revertants of *COXII* in diploids (derived from the cross of the C^R_{336} -carrying strain with the leaky strain 777-3A-M5631) were isolated from cells plated on YG medium. One class was derived from chloramphenicol-resistant colonies exhibiting a 'reduced leaky' phenotype, the other from chloramphenicol-sensitive colonies exhibiting 'leaky' growth. RC cells were grown on YG medium until early stationary phase at 30°C. Growth rates were determined as described by Sakai et al. (1991).

Analysis of mitochondrial translation products

Labeling and detection of mitochondrial translation products were according to Haid et al. (1979), and Weiss-Brummer and Hüttenhofer (1989), except that preincubation with chloramphenicol was omitted. Scanning of autoradiograms was performed with an LKB Ultrascan XL.

Isolation of mitochondrial DNA

DNA was isolated as described by Weiss-Brummer and Hüttenhofer (1989).

DNA sequencing

Total mitochondrial DNA was digested with *HaeIII* (Boehringer-Mannheim). A 4.5 kb fragment containing the 3′ part of the mitochondrial 21S rRNA was isolated and cloned in the Bluescript vector (SK$^-$). Sequencing was according to Chen and Seeburg (1985) using a sequencing kit from Pharmacia. Instead of the universal primer four specifically generated primers (P1-P4) were used; these were based on the DNA sequence of the large ribosomal RNA as derived by Sor and Fukuhara (1982); (P1, 5′-G_{1865}-C_{1893}-3′; P2, 5′-C_{2575}-T_{2622}-3′; P3, 3′-C_{2757}-G_{2778}-5′; P4; 3′-C_{3963}-C_{3990}-5′). These oligonucleotides prime at different positions within the 3′ segment of the 21S rRNA gene.

Results

The chloramphenicol-resistant mutations map to the 21S rRNA gene

The selection of chloramphenicol-resistant mutations of mitochondrial origin is detailed in Materials and methods.The genetic linkage of the newly isolated C^R mutations to the known marker C^R_{321}, which maps to domain V of the 21S rRNA (Dujon 1980), was tested by recombinational analysis with strain KL14-4A. Absence of chloramphenicol-sensitive recombinants in these crosses indicated that the newly isolated mutations must lie very close to, or at the same position as C^R_{321}.

The effect of the mutant ribosomes on translation fidelity (reading frame maintenance)

The leaky strain 777-3A-M5631 carrying the mutant *COXII* reporter gene (Fig. 2, row B) was crossed with each antibiotic-resistant mutant (see Materials and methods). In the cross with the chloramphenicol-resistant mutant KL14-777-3A-C1 recombinant colonies arose which showed a sharp reduction in growth on glycerol i.e. a less leaky phenotype (Fig. 2, row B). When KL14-777-3A-C2 was crossed leaky growth remained unaffected. In KL14-777-3A[C3-C5] crosses some unexpected effects were obtained, which however, were shown not to be correlated with the C^R mutation (data not shown). Thus only one chloramphenicol-resistant mutation examined in this work, the C1 allele, shows fidelity effects detectable as altered leakiness. The C2 and C3–C5 alleles have no effect. These results, obtained under uniform nuclear and mitochondrial genotypic conditions indicate that different mutations associated with chloramphenicol resistance in 21S rRNA have clearly distinguishable effects on translational fidelity.

The colonies from the cross with KL14-777-3A-C1 were examined as in the section on crosses in Materials and methods. Without exception, all colonies with 'reduced leaky' growth were chloramphenicol resistant, whereas all 'leaky' colonies were sensitive to chloramphenicol, like the parental strain 777-3A-M5631 (Fig. 2, row C). The C^R mutation in the diploids reverts to chloramphenicol sensitivity; approximately 2 per 1000 C^R diploid colonies revert to C^S. This made it possible to confirm the strict correlation between chloramphenicol resistance and the 'reduced leaky' growth phenotype on the one hand, and chloramphenicol sensitivity and the standard level of 'leaky' growth on the other. More than 20 chloramphenicol-sensitive revertants (confirmed by sequence analysis to be back mutations, see below) tested had all regained the 'leaky' growth phenotype.

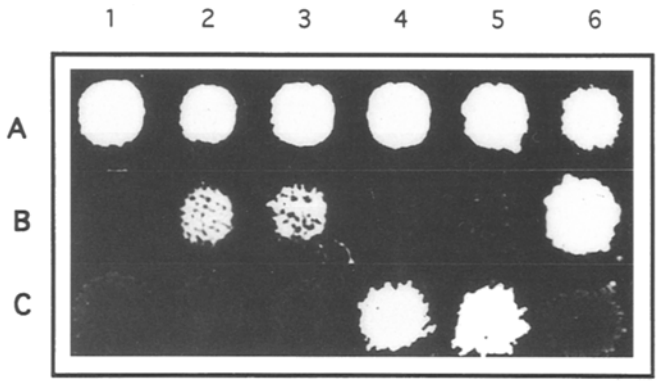

Fig. 2 Phenotypes of strains exhibiting wild-type, leaky, and 'reduced leaky' growth as well as chloramphenicol resistance and sensitivity on selective plates. Colonies from the cross 777-3A-M5631 × KL14-777-3A-C1 (strains 2–6) were collected in micro-titer wells containing YPD, grown to stationary phase, replica-plated onto either glycerol plates (rows A and B) or glycerol plus chloramphenicol (row C) and incubated for 8 days at 30° C. Examples of colony types from the cross are shown. Row **A**: revertants (mit^- to wild-type) of the corresponding strains in row B. For technical reasons (see Materials and methods) these revertants rather than the mutants in row B were tested on chloramphenicol. Row **B**: colonies 2 and 3 are examples of 'leaky' growth, 4 and 5 of 'reduced leaky' growth, and 6 of wild-type growth, Colony 1 is the respiratory-deficient mit^- strain M2511 (column 1). Row **C**: only the 'reduced leaky' phenotype of row B recombinants corresponds with chloramphenicol resistance. No exceptions to this observation were found among large numbers of recombinants

Sequence analysis

The C^R mutation in strain KL14-777-3A-C1 was shown to map close to C^R_{321}, previously localized to domain V of the mitochondrial 21S rRNA gene (Dujon 1980). Domain V sequence analysis was thus performed on the C1 allele as described in Materials and methods. The DNA sequencing showed a single T to C exchange at position 2677. This exchange was also found previously by Dujon and Jacquier (1983), but in a strain non-isogenic to 777-3A, and was designated by them as C^R_{336} (see Fig. 3). The site of C^R_{336} corresponds to position U_{2504} of the central loop in domain V in *E. coli* 23S rRNA. Three further C^R mutations in strain KL14-777-3A (C3–C5) proved to be identical to C^R_{321}, having an A to C alteration at yeast position 2676. 'Leaky' C^S derivatives of $C^R{}_{336}$, as noted above, all showed a C to T exchange at position 2677.

Influence of the chloramphenicol-resistant ribosomes on growth rate

As well as differing in fidelity effects, the various C^R mutations also confer sharply differing growth rates. The resistant haploid strains KL14-777-3A-[C2-C5] grow normally.The resistant haploid strain KL14-777-3A-C1, in contrast to C2–C5, exhibits a reduced growth

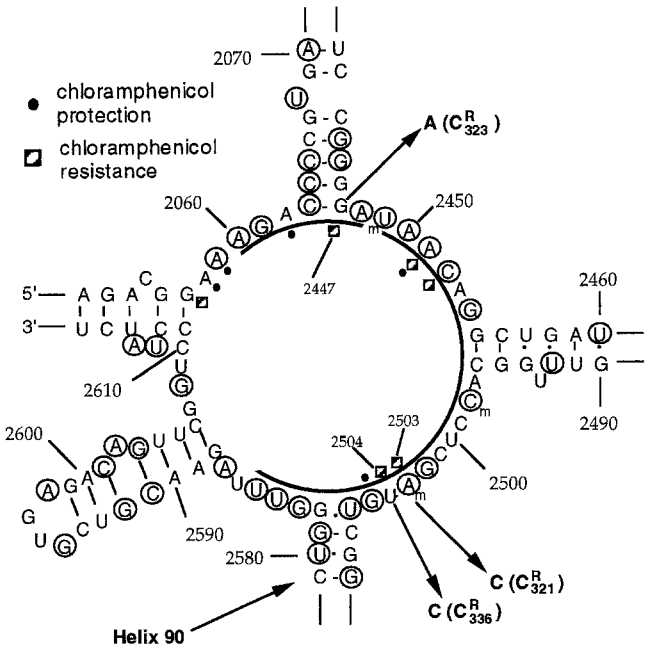

Fig. 3 Secondary structure of the central loop of domain V in *E. coli* 23S rRNA. Encircled nucleotides are completely conserved in 23S-like RNA of 28 organisms including eukaryotes, Archaebacteria and Eubacteria as summarized by Vester and Garrett (1988). Compared to its counterpart in yeast mitochondria, the *E. coli* sequence has five extra bases in the central loop itself, but these are to the left of helix 90; the bases to the right of helix 90 denoted by the *solid arc* are identical in *E. coli* and yeast mitochondria. According to the data of Smith and Cooperman (1993) A_{2503} (equivalent to C^R_{321}) is methylated in *E. coli*. Yeast mitochondrial positions in this paper are numbered according to Dujon and Jacquier (1983). C^R_{336} is at position 2677 (U_{2504} in *E. coli*); C^R_{321} at 2676 (A_{2503} in *E. coli*) and C^R_{323} at 2620 (G_{2447} in *E. coli*). For sites of protection by, and resistance to, chloramphenicol see Noller (1993, Fig. 2 and references therein)

rate detectable under conditions of mitochondrial translation. This reduction in leakiness of the *COXII* mutation in diploids, in the presence of the C1 allele, could be one manifestation of a general reduction in the rate of translation of all mitochondrial genes, rather than being specifically related to frameshifting. These alternative possibilities are distinguishable by studying the effect of the C^R mutation on the production of other mitochondrial proteins, and by examination of growth rate in the presence of the normal *COXII* gene.

The growth rates of two diploid recombinant colonies (from the cross 777-3A-M5631 × KL14-777-3A-C1), both revertant for the + 1 frameshift mutation M5631 and therefore respiratory competent, were found to be indistinguishable when measured under conditions of mitochondrial translation. One recombinant colony carried C^R_{336}, the other the wild-type C^S allele. If the resistance mutation had a general deleterious effect on translation, one would, on the contrary, have expected cells derived from the chloramphenicol-resistant colony to display a comparatively diminished growth rate. Several other independent derivatives were also tested, and in each case the result indicated that, in contrast to the haploid situation, the growth of the diploid carrying C^R_{336} resembles that of wild-type.

This demonstrates that the C^R_{336} mutation has a major effect on ribosomal function only in the tested haploid strain. Similar effects of C^R_{336} in another haploid yeast strain and of some C^R mutations in *E. coli* have been reported (Dujon and Jacquier 1983; Vester and Garrett 1988). Therefore to distinguish between general translation effects of C^R_{336} and its specific action on *COXII* mutants, the effects were studied only in diploids that showed normal growth.

Resistant ribosomes reduce the leakiness of the *COXII* reporter but not the levels of other mitochondrial proteins

Mitochondrial proteins were labelled, in the presence of cycloheximide with radioactive sulphur and resolved on an SDS gradient gel (10–15%) as shown in Fig. 4. There are five major mitochondrial proteins. These are var1 (V), subunits I–III of cytochrome *c* oxidase (I–III) and cytochrome *b* (b). Only the level of subunit II of cytochrome *c* oxidase (*COXII*) is altered. In lane 2 the amount of subunit II expressed reflects the leakiness of the defective *COXII* reporter gene, which, as published previously (Fox and Weiss-Brummer 1980) is estimated to express 20% of wild-type protein level (Weiss-Brummer and Hüttenhofer 1989). Where the ribosomes are chloramphenicol resistant, the level of subunit II is reduced to about 10% of wild type (Fig. 4, lane 1). This value is based on densitometric scanning of the autoradiogram and demonstrates a pronounced effect of the chloramphenicol-resistant mutation on frameshifting. The 'reduced leaky' phenotype can thus be correlated with a reduction in the amount of subunit II.

Effect of the resistant ribosomes on other frameshift, nonsense and missense *COXII* and *COB* mutants

The effect of C^R_{336} on misreading, frameshifting and read-through of nonsense codons was tested upon a set of 12 mit^- mutants, all of which share the same nuclear and mitochondrial composition as 777-3A-M5631 (Table 2). Eleven mutants are not leaky on glycerol and can therefore be tested only for increased growth. They include two examples of + 1 frameshift mutations generating reiterated T sequences (Fig. 5). Diploid cells from crosses between the C^R_{336}–containing strain and these mutants, were replica-plated onto glycerol medium. No increase in growth was observed (see Table 2). In contrast to these mutants, strain 777-3A-M5701 normally exhibits leaky growth on this medium. This strain carries a − 1 T mutation at almost

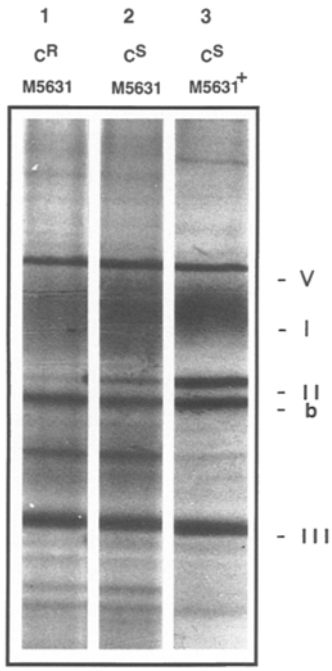

Fig. 4 Analysis of mitochondrial translation products. Cells were labelled in the presence of cycloheximide with radioactive ^{35}S. Mitochondrial proteins were resolved on a 10–15% SDS gradient gel. Lane 1, chloramphenicol-resistant ribosomes, *COXII* (M5631) mutated reporter; lane 2, wild-type (chloramphenicol-sensitive) ribosomes, *COXII* mutated reporter; lane 3, wild-type ribosomes, wild-type *COXII*. The five major mitochondrial proteins are indicated as follows V, var1 (44 kDa); I–III, subunit I–III of cytochrome c oxidase (40 kDa, 33.5 kDa, 23 kDa); b, apocytochrome *b* (30 kDa). The autoradiogram shows the products of three strains. Lanes 1 (a representative of 'reduced leaky' strains) and 2 (a leaky strain) were from the cross KL14-777-3A-C1 × 777-3A -M5631; the wild-type strain in lane 3 was from the cross KL-0 × 777-3A. Only subunit II of cytochrome *c* oxidase is affected. Lane 2 shows the product of the leaky *COXII* reporter gene; the level is estimated as 20% of that of the wild-type in lane 3. This level of leakiness is reduced by approximately 50% in the chloramphenicol-resistant background (lane 1). These values (average of three representatives each of 'reduced leaky' or leaky strains) were obtained by scanning the autoradiogram using the stable, ribosomal var1 protein as an internal standard for the determination of the amount of subunit II for each lane. In lane 1, subunit I of cytochrome c oxidase is also reduced in amount. Previous results (unpublished data) show that it is unlikely that this reduction arises from the chloramphenicol-resistant phenotype. In many other *COXII* mutants, where subunit II of cytochrome c oxidase is defective, a similar reduction in the level of subunit I protein is frequently observed

the same location as 777-3A-M5631 (Fig. 1). Its leakiness was clearly reduced (Table 3) when, following crosses with the C^R_{336}–containing strain, C^R recombinants were compared for growth to the C^S recombinants. This level of reduction was not quantified in a protein gel analysis on account of the low degree of leakiness of the mutant strain 777-3A-M5701 in the first place. The above tests are not extensive, mainly due to the unavailability of other yeast leaky mitochondrial frameshift mutants; nevertheless they permit the tentative conclusion that C^R_{336} in yeast mitochondria does not lower fidelity; on the contrary, apparently increased accuracy is observed under special circumstances.

Discussion

The main finding in this paper is that a mitochondrial chloramphenicol-resistant mutation in yeast, C^R_{336}, mapping to domain V of the 21S rRNA, causes the ribosomes to restrict frameshifting.This is the first example of a C^R mutation with this in vivo effect. C^R_{336} maps to position U_{2677}, a highly conserved base (equivalent to U_{2504} in *E. coli*, Fig. 3) in the central loop of domain V. Some lines of evidence suggest that the central loop forms part of the peptidyltransferase center (Vester and Garrett 1988; Noller 1991; reviewed in Noller 1993).

Apart from the present work, the alteration of one other base, G_{2583} (of *E. coli*) in the domain V central loop has been shown to affect fidelity. Substitutions at this position (tested under in vitro conditions only) increased accuracy in the order G < A < U < C (Saarma and Remme 1992). The exact mechanisms of action of position 2583 mutations are unknown, but chemical probing studies of Moazed and Noller (1989) implicate both of the bases U_{2584} and U_{2585} adjacent to the G_{2583} site as important in P-site 3′ tRNA protection. The only other mutations of 21S/23S rRNA known to affect fidelity (O'Connor and Dahlberg 1993; Melançon et al. 1992) are outside of the central loop of domain V.

In this work the negative effect of ribosomes containing C^R_{336} on translation of mRNAs from two alleles of *COXII* is described. It is pertinent to the translational consequences of C^R_{336} to consider how frameshifting in the *COXII* gene may be achieved in the first place. The mutation M5631 is a + U addition to a wild-type U5 run, generating the mRNA sequence AUU UUU UCC A (Fig. 1). The observed leakiness resulting from this mutation is most probably not primarily based on the intrinsic 'slipperiness' of the U repeat sequence present, but rather is related to the sequence UCC A immediately downstream of the first U5 run. Mutations M4577 and M4931, like M5631, result from + U additions at sequences containing five or six reiterated Us (Fig. 5) but such strains are not leaky for growth on glycerol (Weiss-Brummer et al. 1984). On the other hand the − 1 frameshift mutant 777-3A-M5701 has only 4 Us in tandem, but is leaky (Fig. 1). These facts dissociate leakiness from mutations at U runs per se.

To account for the leakiness (frameshifting) of M5631,we first note two particular observations. Firstly in the wild-type protein proline (see pos. 263–265, Fig. 1) is totally conserved in the *COXII* protein of

Table 2 The effect of the C^R_{336} mutation on frameshifing or misreading of a set of *COXII* and *COB* mutants. The C^R mutant strain KL 14-777-3A-C1 was crossed to a series of mit^- mutants all derived from strain 777-3A. The crosses were performed as described in Materials and methods. +: indicates that the frameshifting rate was reduced; -: indicates no observable effect on frameshifting and no suppression of the nonsense and missense mutants

Mutation	Lesion	Gene	C^R_{336} effect	References[a]
M4611	TTA to TAA (ochre)	*COXII*	–	1
M5611	CAA to TAA (ochre)	*COXII*	–	1
M5801	GGT to GTT (missense)	*COXII*	–	1
M2511	– 1 frameshift	*COXII*	–	1
M5701	– 1 frameshift	*COXII*	+	2
M5631	+ 1 frameshift	*COXII*	+	2
M4577	+ 1 frameshift	*COB*	–	3
M4901	– 1 frameshift	*COB*	–	3
M631	– 1 frameshift	*COB*	–	3
M4931	+ 1 frameshift	*COB*	–	3
M7711	TGT to TAT (missense)	*COB*	–	3
M6821	TAT to TAA (Ochre)	*COB*	–	3

[a] 1, Fox and Staempfli (1982); 2, Fox and Weiss-Brummer (1980); 3, Weiss-Brummer et al. (1984)

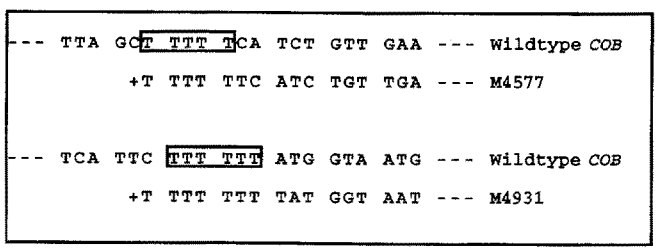

Fig. 5 Sequence alterations caused by two frameshift mutations at reiterated T bases in the *COB* gene. Sequence data are derived from Weiss-Brummer et al. (1984). Reiterated T sequences are boxed in the wild-type sequences. Positions of bases in the boxes are 183–187 (M4577) and 265–270 (M4931) according to Lazowska et al. (1983)

Table 3 Phenotypes of reduced leaky and leaky colonies of M5631 and M5701. The extent of growth on a medium containing a non-fermentable carbon source is designated by (+), + or + + (*see Figure 2B, lane2 and 3 for the extent of + +) in comparison with wild-type growth to which + + + + is assigned. No growth is designated by –. Respiratory-deficient cells cannot grow on a medium containing glycerol as the only carbon source

Phenotypes	Growth at 23° C	30° C	36° C
Respiratory-deficient	–	–	–
Reduced leaky (M5701)	–	–	(+)
Leaky (M5701)	–	+	+
Reduced leaky (M5631)	–	(+)	+
Leaky (M5631)	–	+ + *	+ +
Wild-type	+ + + +	+ + + +	+ + + +

different organisms, indicating a crucial functional role for this residue (see Sakai, 1989). Therefore, during decoding at the sequence AUU UUU UCC A in M5631, the only possible competitive mispairing at the A-site UCC with the potential to produce a fruitfully frameshifted product would be with the $tRNA^{pro}$(3′GGU5′). Other mispairings can be discounted by the proline conservation constraint. Secondly, in the wild-type mitochondrial protein gene UCC is only used once and its cognate tRNA (3′AGU5′) may decode it inefficiently. This may mean that the rarely utilized codon UCC may trigger a ribosomal stall (see Sørensen et al. 1989; Dix et al. 1990; Varenne et al. 1982 for example of rare codons resulting in poor translatability and ribosomal pausing).

On these bases, three models are presented in Table 4 to account for frameshifting in 777-3A-M5631. Model A is reminiscent of + 1 frameshifting in the yeast transposable element Ty3 (Farabaugh et al. 1993) and model B of + 1 frameshifting in the yeast transposable elements Ty1 and Ty2 (Belcourt and Farabaugh 1990). For the record, no simultaneous tRNA shifting (model C) has been reported so far for + 1 frameshifting.

The other leaky (but less so) mutant 777-3A-M5701 requires a leftward shift to restore the zero reading frame (Fig. 1). It generates a CAG glutamine codon in the sequence AUU UUC CAG; this codon is also (extremely) rare (4 instances in the entire set of mitochondrial genes). The proline conservation constraint suggests that $tRNA^{pro}$(3′GGU5′) must read its own cognate codon in the – 1 frame UU UUC CAG, resumably following its rejection and a stall at the CAG in the A-site (see model D). Experiments by Kolor et al. (1993) and Gallant and Lindsley (1992) strongly suggest that a leftward shifted entry to the A-site is only successful when the P-site tRNA can recouple to the left also and allow access to its codon's wobble base. Retroviral frameshift sites (e.g. Jacks et al. 1988; see Hatfield et al. 1992; Brierley et al. 1992) provide striking examples of this forced P-site tRNA recoupling. It is therefore more likely that a double (the potential for which exists), rather than a single, shift is involved in M5701

C^R_{336} significantly affects the level of the frameshifted product of *COXII* but has no detectable effect on the wild-type products of other mitochondrial genes (Fig. 4). This suggests that the altered C^R_{336} domain V may only behave differently from its wild-type counterpart when an abnormality in decoding triggers a ribosomal stall or promotes mispairing. We note that immediately 3′ to the frameshift site in *COXII* the potential for the formation of a weak stem–loop configuration exists (underlined in Fig. 1). Such a structure, if

Table 4 Summary of models (A–D) for mechanisms of frameshift restriction by C^R_{336}. For further details, see Discussion

Mutant	tRNA(s) that cause a shift	Frameshifting events in C^S ribosomes leading to 20% wild-type product in M5631	Frameshifting events in C^R ribosomes leading to 10% wild-type product in M5631
		P-site|A-site AUU UUU|UCC A phe ser	
M5631 **A**	pro	Vacancy of **A**-site due to rare usage of UCC serine ⇒ opportunistic binding of pro tRNA one base to the right ⇒ **P**-site and **A**-site tRNAs spatially abnormal, but tolerable transpeptidation rate ⇒ decoding in the zero frame	**P**- and **A**- site tRNA spatially or conformationaly even more abnormal ⇒ lower transpeptidation rate ⇒ lower rate of zero frame decoding
B	phe	Vacancy of **A**-site due to rare usage of UCC serine destabilizes phe tRNA in **P**-site ⇒ phe re-engagement one base to the right ⇒ decoding in the zero (wild-type) frame	Slower release of phe tRNA from the **P**-site allows time for serine tRNA to engage at UCC ⇒ lower rate of zero frame decoding
C	phe-pro	Mispairing at the **A**-site between pro tRNA and UCC serine codon ⇒ destabilization of both **P**- and **A**-site tRNAs which each re-engage one base to the right ⇒ wild-type (zero) frame decoding	Slower release of the phe tRNA from the **P**-site allows longer proofreading time for rejection of pro tRNA from UCC serine ⇒ reduced rate of simultaneous tRNA shift and zero frame decoding
		P-site|A-site AUU UUC|CAG phe gen	
M5701 **D**	phe-pro	Mispairing at the **A**-site between pro tRNA and CAG glutamine codon ⇒ destabilization of both P site- and A-site tRNAs ⇒ each re-engaging one base to the left ⇒ wild-type (zero) frame decoding	Slower release of the phe tRNA from **P**-site allows longer proof reading time for rejection of pro tRNA from CAG glutamine codon ⇒ reduced rate of simultaneous tRNA shift and of zero frame decoding

formed, could either add to the decoding difficulties at CAG in M5701 (or UCC in M5631) or possibly serve as the determinant for a ribosomal stall. In that case the main function of the C^R_{336} alteration could possibly be the modulation of this stem-loop structure. However, we believe that the structural weakness of this potential configuration precludes its consideration as the sole or main determinant of ribosomal stalling. It is more likely that C^R_{336} affects transpeptidation or rRNA/tRNA association-dissociation.

Some lines of evidence (Hall et al. 1988; Steiner et al. 1988) imply that A-site bound tRNA may crosslink to the equivalent of the C^R_{336} site (U_{2504}) and some nearby bases in the *E. coli* domain V central loop. In addition, the C^R_{336} equivalent is immediately adjacent to two bases, G_{2505} and U_{2506}, known in *E. coli* to be sites protected by tRNA when tRNAs are bound to the ribosomal P-site (Moazed and Noller, 1989). U_{2506}, together with the structurally adjacent U_{2584} and U_{2585} is protected by the 3′ end of P-site tRNA (Moazed and Noller, 1991). There is therefore some reasonable ground for linking C^R_{336} with effects at the A- and P-sites.

One alternative for +1 frameshifting (Model A, Table 4) entails a single skipped base separating the A- and P-site tRNAs on the mRNA in the 30S subunit. This situation would arise specifically at the frameshift sequence. In this case inefficiency of transpeptidation in C^R relative to C^S ribosomes at each round of translation of *COXII* could result in less leaky product per unit time (Model A, Table 4, right column), i.e. in this model C^R_{336} would act at the level of transpeptidation.

On the other hand could reduced frameshifting involve a direct effect of C^R_{336} at the A-site? Although it is formally possible that C^R ribosomes could reduce frameshifting by accepting a near-cognate tRNA at the A-site more often than C^S ribosomes (i.e. affect proofreading), the location of C^R_{336} within domain V raises some difficulties in this regard. According to Moazed and Noller (1989) the EF-Tu ternary complex shields the interaction between tRNA and domain V bases up to the time of its (EF-Tu.GDP) dissociation from the

ribosome. On present understanding therefore, by the time the A-site tRNA interacts with domain V, proofreading is already completed, i.e. it is unlikely that C^R_{336} directly affects tRNA interaction at the A-site.

Finally could C^R_{336} reduce frameshifting by directly affecting tRNA interaction at the P-site? One possibility that was discussed for + 1 frameshifting involved abnormal dissociation of P-site tRNA and its subsequent re-engagement one base to the right before filling of the A-site and transpeptidation (Model B, Table 4). In considering this model and its variant (Model C, Table 4), it is conceivable that the mutation in C^R_{336} could be silent with respect to normal P-site dissociation but 'active' when dissociation is premature, i.e. when dissociation occurs before off-loading of the nascent peptide. A conformationally altered binding of peptide-bearing P-site tRNA, could, by effectively suppressing premature dissociation, also reduce the level of the leaky product. An altered tRNA binding effect of C^R_{336} at the P-site might also adversely influence a leftward shift in − 1 frameshifting in mutant M5701 (Model D, Table 4, right column) in a manner analogous to that just described. Irrespective of the specific mechanism, the overall effect in the above models is equivalent to increased translational accuracy due to frame maintenance at particular mRNA stall sites, without an accompanying effect on normal translation.

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References

Allen PN, Noller HF (1989) Mutations in ribosomal proteins S4 and S12 influence the higher order structure of 16S ribosomal RNA. J Mol Biol 208: 457–468

Allen PN, Noller HF (1991) A single base substitution in 16S ribosomal RNA suppresses streptomycin dependence and increases the frequency of translational errors. Cell 66: 141–148

Atkins JF, Weiss RB, Gesteland RF (1990) Ribosome gymnastics – degree of difficulty 9.5, style 10.0. Cell 62: 413–423

Atkins JF, Gesteland R (1994) Skipping base(s) or braking an initial engagement and repairing to a new triplet codon are involved in natural frameshifting and hopping. In: Söll D, Rajbhandary U (eds) Transfer RNA. American Society for Microbiology, Washington DC, pp 471–490

Ballesta JPG, Lazaro E (1990) Peptidyltransferase inhibitors: structure-activity relationship analysis by chemical modification. In: Hill WE, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR (eds) The ribosome: structure, function and evolution. American Society for Microbioliology, Washington DC, pp 502–510

Belcourt MF, Farabaugh PJ (1990) Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage of a 7-nucleotide minimal site. Cell 62: 339–352

Brierley I, Digard P, Inglis SC (1989) Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. Cell 57: 537–547

Brierley I, Jenner AJ, Inglis SC (1992) Mutational analysis of the 'slippery-sequence' component of a coronavirus ribosomal frameshifting signal. J Mol Bio. 227: 463–479

Celma ML, Monro RE, Vazquez D (1970) Substrate and antibiotic binding sites at the peptidyltransferase centre of *E. coli* ribosomes. FEBS Lett 6: 273–277

Chamorro M, Parkin N, Varmus HE (1992) An RNA pseudoknot and an optimal heptameric shift site are required for highly efficient ribosomal frameshifting on a retroviral messenger RNA. Proc Natl Acad Sci USA 89: 713–717

Chen E, Seeburg PH (1985) Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA 4: 165–170

Dix DB, Thomas LK, Thompson RC (1990) Codon choice and gene expression: synonymous codons differ in translational efficiency and translational accuracy. In: Hill WE, Dahlberg A, Garrett RA, Moore PB, Schlessinger D, Warner JR (eds) The ribosome: structure, function and evolution. American Society for Microbiology, Washington DC, pp 527–533

Dujon B (1980) Sequence of the intron and flanking exons of the mitochondrial 21S rRNA gene of yeast strains having different alleles at the *ω* and *rib*-1 loci. Cell 20: 185–197

Dujon B, Jacquier A (1983) Organization of the mitochondrial 21S rRNA gene in *Saccharomyces cerevisiae*: mutants of the peptidyltransferase centre and nature of the omega locus. In: Schweyen RJ, Wolf K, Kaudewitz F (eds) Mitochondria 1983. Nucleo-mitochondrial interactions. De Gruyter, Berlin, pp 389–403

Farabaugh PJ, Zhao H, Vimaladithan A (1993) A novel programed frameshift expresses the *POL3* gene of retrotransposon Ty3 of yeast: frameshifting without tRNA slippage. Cell 74: 93–103

Fox TD (1979) Five TGA 'stop' codons occur within the translated sequence of the yeast mitochondrial gene for cytochrome c oxidase subunit II . Proc Natl Acad Sci USA 76: 6534–6538

Fox TD, Weiss-Brummer B (1980) Leaky + 1 and − 1 frameshift mutants at the same site in a yeast mitochondrial gene. Nature 288: 60–63

Fox TD, Staempfli S (1982) Suppressor of yeast mitochondrial ochre mutations that maps in or near the 15S rRNA gene of mitochondrial DNA. Proc Natl Acad Sci USA 79: 1583–1587

Gallant JA, Lindsley D (1992) Leftward ribosome frameshifting at a hungry codon. J Mol Biol 223: 31–40

Gesteland RF, Weiss RB, Atkins JF (1992) Recoding: reprogrammed genetic decoding by special sequences in mRNAs. Science 257: 1640–1641

Haid A, Schweyen RJ, Bechmann H, Kaudewitz F, Solioz M, Schatz G (1979) The mitochondrial *COB* region in yeast codes for apocytochrome *b* and is mosaic. Eur J Biochem 94: 451–464

Hall CC, Johnson D, Cooperman BS (1988) [^{3}H]-*p*-Azidopuromycin photoaffinity labeling of *Escherichia coli* ribosomes: evidence for site-specific interaction at U-2504 and G-2502 in Domain V of 23S ribosomal RNA. Biochemistry 27: 3983–3990

Hatfield DL, Levin JG, Rein A, Oroszlan S (1992) Translational suppression in retroviral gene expression. Adv Virus Res 41: 193–239

Huang WM, Ao S-Z, Casjens S, Orlani R, Zeikus R, Weiss R, Winge D, Fang M (1988) A persistent untranslated sequence within bacteriophage T4 DNA topoisomerase gene 60. Science 239: 1005–1012

Jacks T, Power MD, Masiarz PP, Luciw P, Bar PJ, Varmus HE (1988) Characterization of ribosomal frameshifting in HIV *gag-pol* expression. Nature 331: 280–283

Kawakami K, Pande S, Faiola B, Moore DP, Boeke JD, Farabaugh PJ, Strathern JN, Nakamura Y, Garfinkel DJ (1993) A rare tRNA-Arg(CCU) that regulates Ty1 element ribosomal frameshifting is essential for Ty1 retrotransposition in *Saccharomces cerevisiae*. Genetics 135: 309–320

Kirsebom LA, Isaksson LA (1985) Involvement of ribosomal protein L7/L12 in control of translational accuracy. Proc Natl Acad Sci USA 82:717–721

Kolor K, Lindsley D, Gallant JA (1993) On the role of the P-site in leftward ribosomal frameshifting at a hungry codon. J Mol Biol 230:1–5

Kotylak Z, Slonimski PP (1977) Mitochondrial mutants isolated by a new screening method based upon the use of the nuclear mutation *op*1. In: Bandlow W, Schweyen RJ, Wolf K, Kaudewitz F (eds) Mitochondria 1977: Genetics and biogenesis of mitochondria. De Gruyter, Berlin, pp 83–89

Lancashire WE, Mattoon JR (1979) Cytoduction: a tool for mitochondrial genetics studies in yeast. Mol Gen Genet 170:333–344

Lazowska J, Gargouri A, Slonimski PP (1983) The corrected sequence of the first intron of the *cob-box* gene in the yeast strain 777–3A. In: Schweyen RJ, Wolf, K Kaudewitz F (eds) Mitochondria 1983. De Gruyter, Berlin, pp 405–410

Le S-Y, Chen J-H, Maizel JV (1993) Identification of unusual RNA folding pattern encoded by bacteriphage T4 gene 60. Gene 124:21–28

Melançon P, Tapprich WE, Brakier–Gingras L (1992) Single base mutations at the position 2661 of *Escherichia coli* 23S rRNA increase efficiency of translational proofreading. J Bacteriol:174:7896–7901

Moazed D, Noller HF (1987) Chloramphenicol, erythromycin, carbomycin and vernamycin B protect overlapping sites in the peptidyltransferase region of 23S ribosomal RNA. Biochimie 69: 879–884

Moazed D, Noller HF (1989) Interaction of tRNA with 23S rRNA in the ribosomal A, P, and E sites. Cell 57:585–597

Moazed D, Noller HF (1991) Sites of interaction of the CCA end of the peptidyl–tRNA with 23S rRNA. Proc Natl Acad Sci USA 88:3725–3728

Monro RE, Vazquez D (1976) Ribosome-catalysed peptidyl transfer: effects of some inhibitors of protein synthesis. J Mol Biol 28:161–165

Noller HF (1991) Ribosomal RNA and translation. Annu Rev Biochem 60:191–227

Noller HF (1993) Peptidyl transferase: protein, ribonucleoprotein, or RNA J Bacteriol 175:5297–5300

O'Connor M, Göringer HU, Dahlberg AE (1992) A ribosomal ambiguity mutation in the 530 loop of *E. coli* 16S rRNA. Nucleic Acids Res 20:4221–4227

O'Connor M, Dahlberg AE (1993) Mutations at U2555, a tRNA–protected base in the 23S rRNA, affect translational fidelity. Proc Natl Acad Sci USA 90:9214–9218

Parker J (1989) Errors and alternatives in reading the universal genetic code. Microbiol Rev 53:273–298

Parkin NT, Chamorro M, Varmus HE (1992) Human immunodeficiency virus type 1 *gag -pol* frameshifting is dependent on downstream mRNA secondary structure: demonstration by expression in vivo. J Virol 66:5147–5151

Prescott CD, Dahlberg AE (1990) A single base change at 726 in 16S rRNA radically alters the pattern of proteins synthesized in vivo. EMBO J 9:289–294

Saarma U, Remme J (1992) Novel mutants of 23S rRNA: characterization of functional properties. Nucleic Acids Res 20: 3147–3152

Sakai H (1989) Suppression einer mitochondrialen Frameshift-Mutation der Hefe *S. cerevisiae*. Thesis, Universität München

Sakai H, Stiess R, Weiss-Brummer B (1991) Mitochondrial mutations restricting spontaneous translational frameshift suppression in the yeast *Saccharomyces cerevisiae*. Mol Gen Genet 227:306–317

Smith JE, Cooperman BS (1993) Methylation sites in *Escherichia coli* ribosomal RNA: localization and identification of four new sites of methylation in 23S rRNA. Biochemistry 31: 10825–10834

Sørensen MA, Kurland CG, Petersen S (1989) Codon usage determines translation rate in *E. coli*. J Mol Biol 207:365–377

Sor F, Fukuhara H (1982) Complete DNA sequence coding for the large ribosomal RNA of yeast mitochondria. Nucleic Acid Res 11:339–348

Steiner G, Kuechler E, Barta A (1988) Photo–affinity labelling at the peptidyl tranferase centre reveals two different positions for A- and P-sites in domain V of 23S rRNA. EMBO J 7: 3949–3955

Ten Dam E, Pleij CWA, Bosch L (1990) RNA pseudoknots: translational frameshifting and read-through on viral RNAs. Virus Genes 4:121–136

Tu C, Tzeng TH, Bruenn J (1992) Ribosomal movement impeded at a pseudoknot required for frameshifting. Proc Natl Acad Sci USA 89:8636–8640

Varenne S, Knibiehler M, Cavard D, Morlon J, Lazdunski C (1982) Variable rates of polypeptide chain elongation for colicins A, E2 and E3. J Mol Biol 159:57–70

Vester B, Garrett RA (1988) The importance of highly conserved nucleotides in the binding region of chloramphenicol at the peptidyltransferase centre of *Escherichia coli* 23S ribosomal RNA. EMBO J 7:3577–3587

Weiss RB, Huang WM, Dunn DM (1990) A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage gene 60. Cell 62:117–126

Weiss–Brummer B, Hüttenhofer A (1989) The paromomycin resistance mutation (par^r 454) in the 15S rRNA gene of the yeast *Saccharomyces cerevisiae* is involved in ribosomal frameshifting. Mol Gen Genet 217:362–369

Weiss–Brummer B, Guba R, Haid A, Schweyen RJ (1979) Fine structure of *oxi1*, the mitochondrial gene coding for subunit II of yeast cytochrome *c* oxidase. Curr Genet 1:75–83.

Weiss–Brummer B, Hüttenhofer A, Kaudewitz F (1984) Leakiness of termination codons in mitochondrial mutants of the yeast *Saccharomyces cerevisiae*. Mol Gen Genet 198:62–68

Wolf K, Dujon B, Slonimski PP (1973) Mitochondrial genetics. V. Multifactoral crosses involving a mutation conferring paromomycin–resistance in *Saccharomyces cerevisiae*. Mol Gen Genet 125 :53–90

De Zamaroczy M, Bernardi G (1986) The primary structure of the mitochondrial genome of *Saccharomyces cerevisiae* — a review. Gene 47:155–177