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# SURVEY AND SUMMARY

# Universal rules and idiosyncratic features in tRNA identity

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## ABSTRACT

**Correct expression of the genetic code at translation is directly correlated with tRNA identity. This survey describes the molecular signals in tRNAs that trigger specific aminoacylations. For most tRNAs, determinants are located at the two distal extremities: the anticodon loop and the amino acid accepting stem. In a few tRNAs, however, major identity signals are found in the core of the molecule. Identity elements have different strengths, often depend more on $k_{cat}$ effects than on $K_m$ effects and exhibit additive, cooperative or anti-cooperative interplay. Most determinants are in direct contact with cognate synthetases, and chemical groups on bases or ribose moieties that make functional interactions have been identified in several systems. Major determinants are conserved in evolution; however, the mechanisms by which they are expressed are species dependent. Recent studies show that alternate identity sets can be recognized by a single synthetase, and emphasize the importance of tRNA architecture and anti-determinants preventing false recognition. Identity rules apply to tRNA-like molecules and to minimalist tRNAs. Knowledge of these rules allows the manipulation of identity elements and engineering of tRNAs with switched, altered or multiple specificities.**

## HISTORICAL BACKGROUND

Transfer RNAs are the interface between DNA encoded genetic information and its expression in proteins. Once Crick proposed the 'adapter hypothesis' (1), tRNAs were discovered 3 years later (2). This led to a desire to understand how the amino acid activating enzymes, the aminoacyl-tRNA synthetases (aaRS), developed (3) and how they recognize tRNAs (4). The challenge was to decipher the recognition sites on the tRNAs which allow unique reading of the genetic code. This pivotal problem in biology was long referred to as a recognition problem and is now known as the tRNA identity problem. The term 'identity' dates back to 1965 (5), and identities were tackled by a variety of methods. These include enzymatic dissection and chemical modification of tRNAs, sequence comparison of isoacceptor tRNAs including mischarged tRNAs and the genetics of suppressor tRNAs (6–8). No unified picture emerged from the early work and tRNA aminoacylation systems appeared idiosyncratic. Nonetheless, attempts were made to find links between them as in the proposal of a discriminator site at N73 (9) and its conservation among molecules charged or mischarged by a same synthetase (7). The idea of a second genetic code was also discussed (4,10,11); it implies conservation of recognition signals in evolution and was rejuvenated (12–14) after the explicit deciphering of the Ala identity (15,16).

Because anticodon nucleotides specify the relationship between an amino acid and a trinucleotide of the genetic code, they were considered privileged candidates to specify recognition by the synthetases. The first experimental fact supporting this view dates to 1964. Kisselev's group showed inactivation of yeast $tRNA^{Val}$ upon chemical modification of its anticodon, an assumption confirmed in Bayev's laboratory (reviewed in 17) using dissected molecules. Evidence for a role of the anticodon in the acceptor function of other tRNAs was obtained for Gly, Met, Phe and Trp specificities. For several others (Glu, Gln, Tyr) the likelihood of anticodon participation in synthetase recognition was high (17). On the other hand, the region near the amino acid $-CCA_{OH}$ accepting end was another obvious recognition candidate. Support for this expectation came from Chambers' laboratory as they used a dissected tRNA to demonstrate a recognition signal for yeast AlaRS embedded in the $tRNA^{Ala}$ acceptor stem, likely within its three first base pairs (6).

An important aspect in all discussions on tRNA identity is the link between specific tRNA–aaRS complex formation and catalysis leading to tRNA aminoacylation. Studies of unspecific tRNA–aaRS interactions and comparisons of cognate tRNA charging with tRNA mischarging led to the concept of kinetic specificity. Correct tRNA aminoacylation is governed more by $k_{cat}$ effects than by $K_m$ effects (7). Specific complex formation is accompanied by conformational changes of the interacting partners (18,19). In a broader perspective, specificity of tRNA aminoacylation relies also on that of the amino acid activation step, on correction mechanisms and on the balance between the concentrations of tRNAs and synthetases that favor cognate aminoacylation and disfavor mischarging (20,21). Discovery in the early 1970s, of tRNA-like molecules (22) that differ in sequence from canonical tRNAs but are substrates of synthetases

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was puzzling. These molecules include structures present at the 3′ extremities of plant viral RNAs (23) as well as other mimics more recently discovered (24–27). A global description of tRNA identity has to integrate all these facts. Additional information on early literature has been reviewed (6,17,28–31).

## DEFINITIONS, GENERAL CONCEPTS AND PREREQUISITES

It is a general belief that tRNA identity is governed by positive (determinants) and negative (anti-determinants) elements that respectively trigger specific aminoacylation and prevent false charging. In each tRNA, positive elements are limited in number and constitute the identity sets. They include isolated nucleotides in single-stranded regions, base pairs in helices, and can also be structural motifs. *A priori*, conserved and semi-conserved residues responsible for tRNA architecture are not part of identity sets. This is, however, not a general rule. In the literature the term 'identity element' often designates elements found *in vivo*, while the term 'recognition element' refers to elements determined *in vitro*. For simplicity we utilize the terminology identity element for both *in vivo* and *in vitro* data. Also, one has to distinguish between 'major' and 'minor' elements. The distinction is not easy and is often subjective. In this survey we consider as major elements those which, upon mutation, lead to the strongest functional effects, either when tested *in vitro* (strongest decrease in aminoacylation efficiency) or *in vivo* (strongest suppression effects). In contrast, minor elements have moderate effects and tune specificity. These elements can act by indirect mechanisms. Altogether, tRNA structure can be considered as a scaffold that permits proper presentation of identity signals to synthetases. After amino acid activation by a synthetase, the catalytic site of the enzyme will be completely switched on by the correct interaction with the tRNA, and tRNA charging can proceed. The implication, *a priori*, is that different types of RNA scaffolds can achieve functional activation of synthetases.

Although this classical view on identity accounts for many of the observed effects, it was recently disturbed with unexpected discoveries such as the existence of alternate identity sets, permissive elements and cryptic determinants (see below). In fact, expression of identities is more subtle than was anticipated and appears to be an intricate interplay of molecular events where mutual adaptation of tRNA and synthetase play a crucial role.

Several technical breakthroughs were essential for deciphering tRNA identities. Methods using RNA polymerases for the *in vitro* transcription of tRNA genes permit preparation of almost all kinds of mutants. The SP6 RNA polymerase was first used for preparation of functional tRNA-like domains from plant viral RNAs (32) and soon after, Uhlenbeck and colleagues popularized the use of T7 RNA polymerase for the systematic synthesis of tRNA variants (33,34). The transcriptional methods yield tRNAs deprived of modified nucleotides, which can be a drawback if epigenetic modifications on native tRNAs play a role in identity. In that case, chemical synthesis of RNA can provide molecules with modified residues. The search for identity elements *in vivo* took advantage of suppressor genetics. A reporter system utilizes a dihydrofolate reductase (DHFR) gene with an amber mutation at position 10 that can be read by any engineered suppressor tRNA provided it is recognized by one or several synthetases (35). Characterization of the amino acids incorporated in DHFR at position 10 gives then direct information on the identity(ies) of the suppressor tRNA. The drawback is that most anticodon identity elements cannot be checked by this methodology. An alternate system using mutants of initiator $tRNA^{Met}$ with unusual anticodon sequences removes this drawback (36,37). A difficulty is the choice of the mutations to introduce in a tRNA. For experimental simplicity and because of the great combinatorial diversity of possible variants, workers in the field have essentially studied single mutants. Doing so, possible combinations of nucleotides that could have functional significance escape detection. Recent studies have shown that such combinations actually exist (see below).

Depending on how identity is achieved, it follows that specificity of a tRNA can be changed by transplantation of its identity set into another tRNA background. Transplantations help to verify completion of identity sets. Sets are considered as complete if their transplantation yields molecules as active as the wild-type tRNA. But caution is required in interpretation, since determinants can escape detection if present in the host tRNA in which functionality of the putative identity set is assayed. *In vivo*, one might consider that mutations in a suppressor tRNA that yield 100% incorporation of a given amino acid in the reporter protein, fully define the identity set for this amino acid specificity. Identity is not defined by physical–chemical properties, but by functional criteria where the balance between correct recognition by one synthetase and non-recognition by the other synthetases play key roles. Also, the *in vivo* approach does not allow screening of all possible tRNA variants with the potential to interact with a synthetase. A mutation introduced in a suppressor tRNA can affect the recognition of this tRNA by other partners of the translational machinery. Identity sets defined according to *in vitro* or *in vivo* criteria, therefore, are not necessarily identical.

Different aspects of tRNA identity have been reviewed in literature (31,38–47). Here we discuss the body of available data in an attempt to uncover universal rules and to understand idiosyncratic features in aminoacylation systems. Deciphering identities relies on functional studies of engineered tRNAs and their understanding is dependent upon advances in structural biology. Thus, the accumulation of tRNA sequences (48) and a better knowledge of the structure of tRNAs (31,49,50), synthetases and their complexes with tRNA (51–60) were pivotal in perceiving interrelations between aminoacylation systems within a given phylum and across phylogenetic lines as well as to understand the origin of tRNA identities.

## IDENTITY ELEMENTS

A compilation of identity elements found to date in cytosolic tRNAs, arranged according to the classification of synthetases in two classes (61,62) and their division into subgroups (55,56), is given in Table 1. Identity elements have been determined for the 20 aminoacylation systems from *Escherichia coli*, 14 systems from *Saccharomyces cerevisiae*, four systems from *Thermus thermophilus* and a few systems from other organisms, often higher eukaryotes including humans. They are in most cases standard nucleotides. Modified nucleotides are identity elements in tRNAs specific for Ile, Glu and Lys in *E.coli* and Ile in yeast. These modifications are exclusively located in the anticodon loop. The scarce involvement of modified nucleotides in tRNA aminoacylation is fortunate and explains why the wide use of unmodified transcripts for deciphering identities by the *in vitro* approach was successful.

**Table 1.** Identity elements in tRNAs aminoacylated by class I (A) and class II (B) synthetases

| A | | *E.coli* [1] | *S.cerevisiae* [2] | *T.thermophilus* [3] | Others [4] | Refs. | Comments |
|---|---|---|---|---|---|---|---|
| **Val** | (a) • | **A73** | **A73** | | | **1.** (64-66) | **2.** proposed on the basis of early biochemical |
| | • | **G3:C70,U4:A69** | - | | | **2.** (17,67,68) | investigations and confirmed on TYMV |
| | (b) • | A35,*C36* | **A35** | — | — | | variants assayed with yeast ValRS |
| | • | - | - | | | | |
| | (c) • | - | - | | | | |
| **Ile** | (a) • | **A73** | - | | | **1.** (64,69-71) | **1.** Lysidine in $tRNA^{Ile}_{II}$, the minor species |
| | • | **C4:G69** | - | | | **2.** (72) | (G34 in $tRNA^{Ile}_{I}$); after removal of $t^6A37$, |
| | (b) • | **L**/G34,A35,U36 | **I34,A35,U36** | — | — | | L=400 |
| | • | **$t^6$A37,A38** | - | | | | **2.** a yeast $tRNA^{Ile}$ isoacceptor species has |
| | (c) • | **U12:A23,C29:G41** | - | | | | Ψ34,A35,Ψ36 |
| **Leu** | (a) • | **A73** | **A73** | | **A73** | **1.** (73,74) | **1.** leucylation is more sensitive to mutations at |
| | • | - | - | | **C3:G70,A4:U69** | **2.** (75) | A14 than U8 in the conserved U8•A14 |
| | | - | - | — | **G5:C68** | **4.** (76,77) | reverse Hoogsteen pair |
| | (b) • | - | **A35** | | - | | **4.** human |
| | • | - | **G37** | | - | | |
| | (c) • | **U8•A14** | - | | **C20a** | | |
| **Met** (fMet) | (a) • | **A73** | **A73** | | | **1.** (78-81) | **1.** nts in brackets are those additionnaly required |
| | • | **(G2:C71,C3:G70)** | - | | | **2.** (82,83) | in initiator tRNA. U4:A69,A5:U68 are |
| | | ***U4:A69,A5:U68*** | - | | | | specific elements in elongator $tRNA^{Met}$ |
| | (b) • | **C34,A35,U36** | **C34,A35,U36** | | | | |
| | • | **(C32,U33,A37)** | **& the 4 other AC** | — | — | | |
| | | - | **loop nts** | | | | |
| | (c) • | - | **D-arm** | | | | |
| **Cys** | (a) • | U73 | **U73** | | | **1.** (84-89) | **1.** the G15•G48 Levitt pair is atypical and |
| | • | *G2:C71,C3:G70* | | | | **2.** (90) | particular to only *E.coli* $tRNA^{Cys}$ |
| | (b) • | G34,*C35,A36* | — | — | — | | |
| | • | - | | | | | |
| | (c) • | **G15•G48,A13•A22** | | | | | |
| **Tyr** | (a) • | A73 | *A73* | | | **1.** (91-94) | **4.** *Pneumocystic carinii* (an eukaryotic |
| | • | - | *C1:G72* | | **C1:G72** | **2.** (95,96) | pathogen). Data obtained from minihelicx |
| | (b) • | U35 | *G34,Ψ35* | — | | **4.** (97) | aminoacylation studies |
| | • | - | - | | | | |
| | (c) • | - | - | | | | |
| **Trp** | (a) • | G73 | - | | G73 | **1.** (98-101) | **4.** *Bacillus subtilis* |
| | • | A1:U72,*G2:C71* | - | | A1:U72, | **2.** (102) | |
| | | *G3:C70* | - | | G5:C68,A9 | **4.** (103) | |
| | (b) • | C34,C35,A36 | C34,C35 | | C34,C35,A36 | | |
| | • | - | - | — | - | | |
| | (c) • | - | - | | - | | |
| **Glu** | (a) • | - | | | | **1.** (35,104-106) | **1.** lack of nt 47 can be considered as an identity determinant, since it alters the stability of the triple interaction (U13•G22)•A46 |
| | • | **G1:C72,U2:A71** | | | | | |
| | (b) • | **$s^4$U34**,U35 | | | | | |
| | • | **A37** | — | — | — | | |
| | (c) • | **U11:A24,** | | | | | |
| | | **U13:G22-A46,Δ47** | | | | | |
| **Gln** | (a) • | **G73** | | | | **1.** (107-110) | |
| | • | **U1:A72**,G2:C71 | | | | | |
| | | G3:C70 | | | | | |
| | (b) • | **Y34,U35,G36** | — | — | — | | |
| | • | **A37,U38** | | | | | |
| | (c) • | **G10** | | | | | |
| **Arg** | (a) • | A/G73 | - | | | **1.** (111-114) | **2.** identity set is different in $tRNA^{Asp}$ transcript |
| | • | - | - | | | **2.** (115) | (see text) |
| | (b) • | **C35,U/G36** | **C35,U/G36** | — | — | | |
| | • | - | - | | | | |
| | (c) • | **A20** | - | | | | |

The distribution of identity nucleotides in tRNA is shown in Figure 1 for the 20 *E.coli* aminoacylation systems, with the distinction between class I and class II synthetases. In both cases, identity elements lie predominantly at the two distal ends of the tRNA, with a strong participation of anticodon residues and discriminator nucleotide N73 together with the most distal base pairs of the amino acid accepting stem. Position 37 in the anticodon loop is only involved in identities of tRNAs charged by class I synthetases. The most distal residues (N73 and the three anticodon nucleotides) are identity elements for most tRNAs. Identity determinants in the tRNA core (nt 8–31 and 39–65) are more system-dependent and are scattered over 21 positions (see the small size of the spheres in Fig. 1A at positions 8, 10–15, 20, 20a, 22–24, 29, 41, 46 and 48, and in B at positions 10, 11, 15, 20, 24, 25, 44, 45, 48, 59 and 60). Such determinants participate in six class I (Ile, Leu, Cys, Glu, Gln and Arg) and three class II (Ser, Pro and Phe) identities. Identity base pairs in anticodon stems were found in four systems (Ile, Ser, Pro and Phe). A few tRNAs with sequence peculiarities utilize them as identity elements: this is the case of the large variable loop in $tRNA^{Ser}$, of residue N-1 in $tRNA^{His}$ and of the atypical G15·G48 Levitt pair in *E.coli* $tRNA^{Cys}$. Altogether, 40 positions have been detected as sites for

**Table 1.** *continued*

| B | | *E.coli* [1] | *S.cerevisiae* [2] | *T.thermophilus* [3] | Others [4] | Refs. | Comments |
|---|---|---|---|---|---|---|---|
| **Ser** | (a) • | *G73* | - | | **G73** | **1.** (73,93,107, | **1.** length of variable region more important than its sequence. Importance of R4:Y69 established on minihelices. G2:C71 is a weak identity element, but acts as an antideterminant against other aaRSs. The eight first listed residues are required to convert a leucine suppressor to a serine suppressor tRNA (73) |
| | • | *C72*, G2:C71, A3:U70, | - | | - | 116-119) | |
| | | *C11:G24*, **R4:Y69** | - | | - | **2.** (120) | |
| | (b) • | - | - | | - | **4.** (76,121) | |
| | • | - | - | — | - | | |
| | (c) • | *C11:G24* | **variable loop** | | **variable** | | |
| | | **variable loop** | | | **loop** | | **4.** human |
| **Thr** | (a) • | - | - | **U73** | | **1.** (122,123) | |
| | • | **G1:C72,C2:G71** | **G1:C72** | **G1:C72,U3:A70** | | **2.** (124) | |
| | (b) • | **G34,G35,U36** | **G35,U36** | **G35,U36** | — | **3.** (125) | |
| | • | - | - | - | | | |
| | (c) • | - | - | - | | | |
| **Pro** | (a) • | **A73** | | | | **1.** (85,126,127) | **4.** human (minor effect of discriminator residue C73) |
| | • | **G72** | | | | **4.** (127a) | |
| | (b) • | **G35,G36** | — | — | **G35,G36** | | |
| | • | - | | | | | |
| | (c) • | **G15•C48** | | | | | |
| **Gly** | (a) • | U73 | **A73** | **U73** | **A73** | **1.** (85,128, | **2.** a yeast $tRNA^{Gly}$ isoacceptor species has G2:T71 (DNA sequence) |
| | • | G1:C72,C2:G71 | **C2:G71,G3:C70** | **G1:C72,C2:G71** | **C2:G71** | 129) | **2 & 3.** nts in bold: with tRNA transcripts from *E.coli* |
| | | G3:C70 | - | **(G3:C70)** | - | **2 & 3.** (130) | **3.** nts in brackets: with tRNA transcripts from *T.thermophilus* |
| | (b) • | C35,**C36** | **C35,C36** | **C35,C36** | - | **3.** (131) | **4.** mammalian |
| | • | - | - | - | - | **4.** (132) | |
| | (c) • | - | - | **(G10:C25)** | - | | |
| **His** | (a) • | C73 | **A73** | | | **1.** (85,129,133- | **1 & 2.** the discriminator base has a moderate effect in yeast, in contrast to *E.coli* where its effect is strong. |
| | • | **G-1** | **G-1** | | | 136) | **1.** importance of anticodon shown by activity of revertant HisRSs on amber $tRNA^{His}$ (134a) |
| | (b) • | *anticodon* | **G34,U35** | — | — | **2.** (137,138) | |
| | • | - | - | | | | |
| | (c) • | - | - | | | | |
| **Asp** | (a) • | **G73** | **G73** | **G73** | | **1.** (139,140) | |
| | • | **G2:C71** | - | - | | **2.** (141,142) | |
| | (b) • | **G34,U35,C36** | **G34,U35,C36** | **G34,U35,C36** | — | **3.** (143) | |
| | • | **C38** | **C38** | **C38** | | | |
| | (c) • | **G10** | **G10•U25** | **G10** | | | |
| **Lys** | (a) • | A73 | | | | **1.** (35,113, | **1.** importance of modified nucleotides: the transcript is 140-fold less efficiently charged than the the native tRNA |
| | • | - | | | | 114) | |
| | (b) • | **U34**,U35,**U36** | — | — | — | | |
| | • | ($\underline{mnm^5s^2U}$)34 | | | | | |
| | (c) • | - | | | | | |
| **Asn** | (a) • | *G73* | | | | **1.** (85,144) | |
| | • | - | | | | | |
| | (b) • | *G34,U35,U36* | — | — | — | | |
| | • | - | | | | | |
| | (c) • | - | | | | | |
| **Phe** | (a) • | *A73* | **A73** | **A73** | **A73** | **1.** (64,145-147) | **4.** human |
| | • | - | - | - | - | **2.** (148-150) | |
| | (b) • | *G34,A35,A36* | **G34,A35,A36** | **G34,A35,A36** | **G34,A35,A36** | **3.** (151,152) | |
| | • | *G27:C43,G28:C42* | $\underline{t^6}$A37 | - | **C30:C40,** | **4.** (153) | |
| | (c) • | *U20,* | **G20** | - | **A31:U39,G20** | | |
| | | *G44,U45,U59,U60* | - | - | - | | |
| **Ala** | (a) • | **A73** | - | | - | **1.** (15,16,129, | **2.** indirect data obtained from aminoacylation studies on dissected tRNA fragments |
| | • | G2:C71,G3•U70 | **G3•U70** | | **G3•U70** | 154-157) | **4.** *Bacillus mori*, rat liver, human & *Arabidopsis thaliana* |
| | | G4:C69 | - | | - | **2.** (158) | |
| | (b) • | - | - | | - | **4.** (154,159) | |
| | • | - | - | — | - | | |
| | (c) • | **G20** | - | | - | | |

The tRNAs are listed according to the synthetase classification in two classes with subclasses. Identity elements are classified according to their location in the amino acid accepting stem (a), anticodon region (b) and other tRNA domains (c). References and comments are given for data concerning *E.coli* (**1**), *S.cerevisiae* (**2**), *T.thermophilus* (**3**) and other organisms (**4**). Identity nucleotides in bold were identified by the *in vitro* approach, those in italics by the *in vivo* approach, and those in normal scripts by both approaches; when underlined, the identity element is the modified nucleotide. Numbering of residues is according to Sprinzl *et al.* (48) and nomenclature of modified nucleotides according to Limbach *et al.* (63). In the case of base pairs, (:) denotes WC pairs, (•) non-WC pairs, and (–) tertiary pairs; (/) indicates that two residues can be identity elements at the same position. R, purine; Y, pyrimidine.

identity signals, including the seven positions of the anticodon loop, N73 and the last 5 bp of the acceptor stem. The remaining base pairs 6–12 of the acceptor branch of tRNA (the continuous acceptor and T-stem helix) are never used in identity, what can be explained *a posteriori*, knowing the contact patterns of tRNAs with synthetases (Fig. 2). In conclusion, classification of identity sets according to the class of the corresponding synthetases is not easily rationalized. It is, however, noticeable that use of position

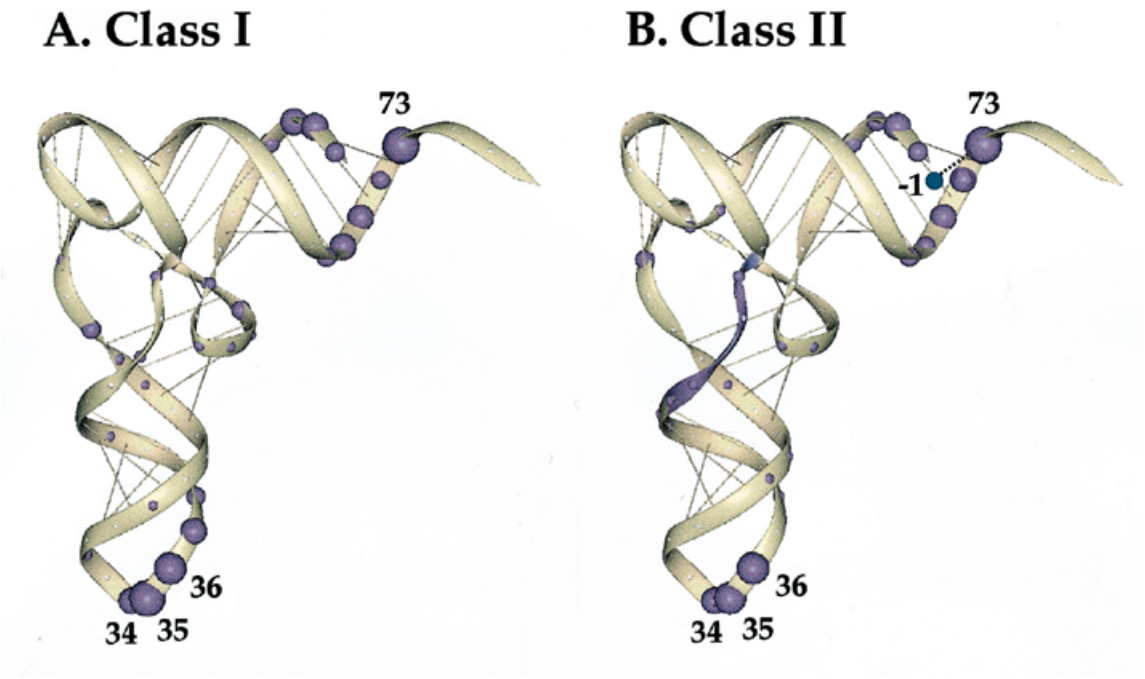


**Figure 1.** Distribution of identity elements for tRNA aminoacylation in the 3D ribbon model of $tRNA^{Phe}$ for tRNAs charged by the 10 class I (**A**) and 10 class II (**B**) synthetases from *E.coli* (drawing with DRAWNA; 160). The size of spheres is proportional to the frequency of identity nucleotides at a given position (five decreasing sizes of purples colored spheres corresponding to 9–10-fold, 7–8-fold, 4–6-fold, 2–3-fold and 1-fold presence of an identity element). (B) The variable domain in purple indicates its participation in Ser identity (in that case this domain is extended to 16 nt).

37 in identity is synthetase class dependent. Also noticeable is the structural rational that emerges when comparing the binding of identity residues to synthetases, which is subclass dependent (see below).

The large occurrence of anticodon and of discriminator residues in identity is emphasized in Table 2. In the 16 *E.coli* systems where anticodon residues contribute to identity, the middle position 35 is always used; positions 36 and 34 are used less often (12- and 11-fold, respectively) with no strict conservation of the identity nucleotide (for Ile, Gln and Arg). Other anticodon loop positions are only sparsely used in identity. Leu, Ser and Ala identities do not rely on anticodon positions. The discriminator position N73 participates in 18 *E.coli* identities, the Glu and Thr identities being the exceptions. Noticeable are the Asp, Lys and Asn identities that specify tRNAs charged by synthetases of subclass IIb; here the discriminator position and the three anticodon nucleotides with conserved U35 are used in the three systems. The similar folding of the anticodon and catalytic domains of the corresponding synthetases accounts for these similarities (55–57). For the known yeast identities, involvement of anticodon and discriminator nucleotides is essentially the same as in *E.coli*, except for the Leu, Arg, His and Ala identities. Opposite to *E.coli*, Leu identity is dependent on residue 35, and Arg and Ala identities do not require the discriminator base. Altogether, out of the 14 yeast identities, 12 (except Ser and Ala) are dependent on the anticodon, and 11 (except Thr) on the discriminator base. A remarkable feature is the phylum-dependent conservation of the discriminator residue in prokaryotic and eukaryotic $tRNA^{Gly}$ and $tRNA^{His}$ species (48) and their probable conserved role in Gly and His identities, as demonstrated in *E.coli* and yeast.

Efficient identity switches of tRNAs with transplanted identity sets are indications for the completion of such sets. Table 3 gives examples where transplantations lead to full activity of the host molecules. In some cases, transplantation of a unique element makes the switch efficient. However, in general, full switches require transplantation of several elements and sometimes engineering of the tRNA scaffold. This is true for switches from $tRNA^{Leu}$ to $tRNA^{Ser}$ and $tRNA^{Phe}$ to $tRNA^{Asp}$ (see below).

## PHENOMENOLOGY OF IDENTITY EXPRESSION

### Strength of identity determinants *in vivo*

The strength of identity determinants expressed *in vivo* is correlated with suppression of a stop codon in a reporter gene. Strength is estimated by the frequency of amino acid incorporation at the suppressed position. Examples are given in Table 4. The amplitude of the effects ranges from 0 to 100% as measured by incorporation of the appropriate amino acid into the reporter protein. Strong effects (>90% non-cognate amino acid incorporation) concern, for example, the A20U mutation in suppressor $tRNA^{Arg}$ which leads to a complete loss of Arg incorporation (thus, A20 is a major Arg identity element). Weaker effects are found, e.g. for the wild-type amber $tRNA^{Arg}$ which has its Arg identity reduced to 37%. This decrease is explained by the presence in $tRNA^{Arg}$ of the amber CUA anticodon which changes the C35 Arg determinant to U35 (Table 1). Interestingly, this tRNA acquires Lys identity (55%) because U35 is a Lys determinant. Mischarging of mutated suppressor tRNAs is common (Table 4). It is explained by the dual effect of the mutations and/or the properties of the suppressor anticodons, which concomitantly withdraw recognition elements for one identity while introducing elements for other identities. Thus, wild-type and mutated suppressor tRNAs often possess multiple identities.

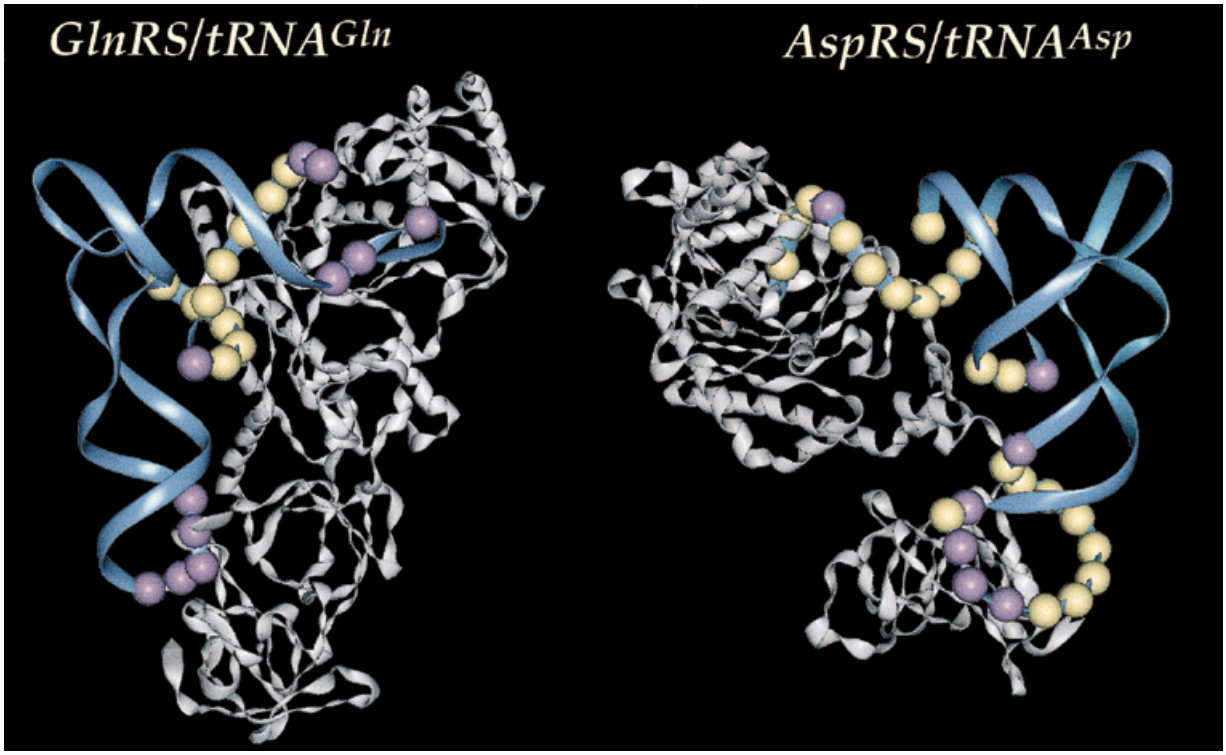


**Figure 2.** Structural comparison of the Gln (left) and Asp (right) tRNA–synthetase complexes highlighting the location of identity elements on the tRNAs and their proximity with the interacting cognate proteins. The figure shows the identity elements (purple spheres) and the non-identity residues (yellow spheres) in contact or close proximity with the synthetases. Coordinates of complexes are from Rould *et al.* (187) and Ruff *et al.* (188), and drawing was produced with DRAWNA (160).

## Strength of identity determinants *in vitro*: $k_{cat}$ effects versus $K_m$ effects

The strategy to measure strength of determinants *in vitro* is completely different to making *in vivo* estimates. Here, one determines intrinsic physical–chemical effects of mutations on aminoacylation reactions instead of evaluating their ultimate fate in protein synthesis. Measurements of loss of kinetic specificities (L) is easy within a wide range, from moderate (L < 10) to important (L > 1000) effects (Table 5). Moreover, the individual contributions of $k_{cat}$ and $K_m$ to L values can be obtained (Table 6). Noticeably, *in vitro* studies seldom report possible mischarging of tRNA variants that are detected *de facto* by the *in vivo* approach.

Contribution of individual nucleotides to identity differs from one tRNA to another and the strength of determinants is phylum-dependent (Table 5). So, the discriminator base is as important (Val), more important (Met and Asp) or less important (Gly and Leu) in prokaryotic than in eukaryotic systems. Furthermore, nucleotides at position 35 are highly represented as identity elements and their alteration produces the same range of effects (Leu, Tyr, Arg, Val and Gly), higher (Trp and Asp) or lower (Phe) effects in prokaryotes than in eukaryotes. No simple picture emerges when comparing prokaryotic and eukaryotic or class I and class II systems. However, the large strength often encountered for N73 and anticodon residues, especially for N35, has to be noticed. Also, in some systems with few identity nucleotides, the relatively low contribution of each residue is noticeable, as in the yeast Phe and Asp systems in which the strongest effects are <1000-fold. In contrast, in the *E.coli* Ala and His systems, also specified by a reduced number of identity residues, effect of their mutation can be >1000-fold.

The relative contributions of $k_{cat}$ and $K_m$ to identity are variable (Table 6). When normalized values are compared (calculated according to a formalism defined in 162) it appears that identity elements produce a continuum of effects ranging from mostly $k_{cat}$ [$(k_{cat})_N > (K_m)_N$] to mostly $K_m$ [$(k_{cat})_N < (K_m)_N$] through mixed $k_{cat}$ and $K_m$ effects [$(k_{cat})_N \sim (K_m)_N$]. Mutations at positions in the vicinity of, or in close contact with synthetases mainly alter $k_{cat}$ suggesting a direct effect on the catalysis of aminoacylation. In contrast, mutation of identity elements involved in tRNA architecture often produce $K_m$ effects. This suggests that these changes modify the functional binding of the tRNA on the enzyme.

Assay conditions influence identity expression. Note the effects of the G73→U73 mutation in *E.coli* $tRNA^{Gln}$ under subsaturating (108) or steady-state kinetic (110) conditions. The influence of $Mg^{2+}$ concentration was mentioned for Phe identity (163). $Mg^{2+}$ concentration modifies the relative strength of identity elements. Also, mutation of the discriminator residue in yeast $tRNA^{His}$ infers moderate (137) or dramatic (138) charging depending on the $MgCl_2$ and ATP concentrations (Table 5).

## Additivity, cooperativity and anti-cooperativity between identity elements

Kinetic data expressed as free energy variation at transition state of multiple mutants and comparison of experimental values with those calculated from data on single mutants define three types of relationships between identity nucleotides. Since such nucleotides

**Table 2.** Involvement of anticodon N34–36 and discriminator N73 nucleotides in tRNA identity

| tRNA identity | anticodon nucleotides | | | | | discriminator nucleotide | |
|---|---|---|---|---|---|---|---|
| | Ec/Sc | | 34 | 35 | 36 | Ec/Sc | 73 |
| **Val** | +/+ | | - | A | - | +/+ | A |
| **Ile** | +/+ | * | L/I | A | U | +/o | A |
| **Leu** | -/+ | * | - | A | - | +/+ | A |
| **Met** | +/+ | * | C | A | U | +/+ | A |
| **Cys** | +/o | | G | C | A | +/+ | U |
| **Tyr** | +/+ | | - | U | - | +/+ | A |
| **Trp** | +/+ | | C | C | - | +/o | G |
| **Glu** | +/o | * | $s^4U$ | U | - | -/o | - |
| **Gln** | +/o | * | C/U | U | G | +/o | G |
| **Arg** | +/+ | | - | C | G or U | +/- | A |
| **Ser** | -/- | | - | - | - | +/o | G |
| **Thr** | +/+ | | - | G | U | -/- | - |
| **Pro** | +/o | | - | G | G | +/o | A |
| **Gly** | +/+ | | - | C | C | +/+ | U/A |
| **His** | -/+ | | G | U | - | +/+ | C/A |
| **Asp** | +/+ | * | G | U | C | +/+ | G |
| **Lys** | +/o | | $mnm^5s^2U$ | U | U | +/o | A |
| **Asn** | +/o | | G | U | U | +/o | G |
| **Phe** | +/+ | | G | A | A | +/+ | A |
| **Ala** | -/- | | - | - | - | +/- | A |

Involvement (+) or non-involvement (–) of N73 and/or anticodon bases in the aminoacylation identity of prokaryotic *E.coli* (Ec) and eukaryotic *S.cerevisiae* (Sc) tRNAs are shown (data originate from *in vitro* and/or *in vivo* studies; Table 1). o, data not available; *, other residues in the anticodon loop are important; L, lysidine; I, inosine. The tRNAs are listed according to the classification of synthetases (Table 1).

are often scattered over three tRNA domains (the anticodon arm, the acceptor arm and the core of the tRNA), the question is how they act together to achieve global specificity. The question is of particular importance if the individual identity residues have moderate strength. Studies of double mutants established that three determinants (anticodon, A73 and G20a) act independently in yeast Phe identity (149). The functional relationship between Asp determinants in yeast $tRNA^{Asp}$ (G73, G34, U35, C36 and G10·U25) was studied with transcripts mutated at two or more identity positions (164). Multiple mutations affect activity of AspRS mainly at the level of $k_{cat}$. Nucleotides located far apart in the 3D structure of the tRNA act cooperatively whereas those of the anticodon triplet act either additively or anti-cooperatively. In the latter case, the effect is lower than the sum of individual effects. These properties are correlated with specific interactions of chemical groups on identity nucleotides with amino acids in the protein as revealed by crystallography (165). Similar effects were found between Asp identity residues in the *T.thermophilus* system (143). Analysis of multiple tRNA mutants in this system revealed cooperativity between determinants of the anticodon loop and G10 and G73. The cooperative effects in the Asp system suggest that conformational changes trigger formation of a functional tRNA–aaRS complex. In yeast, the strongly anti-cooperative triple anticodon mutant indicates that this molecule behaves like an Asp minihelix because it has lost all contacts with the anticodon domain of AspRS. Strikingly, its overall kinetic specificity is identical to that of an Asp minihelix (166).

**Table 3.** Examples of transplantations leading to complete identity switches

| donor $tRNA^x$ | transplanted elements (from x to y) | host $tRNA^y$ | Refs. |
|---|---|---|---|
| *E.coli* $tRNA^{Ala}$ §§ | G3•U70 | $tRNA^{Phe}$, $tRNA^{Cys}$ | (15) |
| yeast $tRNA^{Leu}$ | A73,A35,G37 | $tRNA^{Ser}$ | (75) |
| human $tRNA^{Leu}$ | A73,C3•U70,A4:U69, G5:C68,C20a, var. arm | $tRNA^{Ser}$ | (77) |
| *E.coli* $tRNA^{Met}$ | C34,U35,A36 | $tRNA^{Val}$ (a) | (79) |
| yeast $tRNA^{Met}_i$ | C34,U35,A36,A73 | $tRNA^{Asp}$ | (82) |
| yeast $tRNA^{Phe}$ | G34,A35,A36,G20, G2:C71,G3•U70,A73 α=3,β=2,v=5 | $tRNA^{Asp}$ | (150) |
| *E.coli* $tRNA^{Ser}$ (amber)§ | G73,C72,G2:C71, A3:U70,C11:G24 | $tRNA^{Leu}$ (amber) | (73,116) |
| human $tRNA^{Ser}$ | G73 | $tRNA^{Leu}$ | (76) |
| yeast $tRNA^{Thr}$ | G35, U36 | $tRNA^{Pro}$ | (124) |
| *E.coli* $tRNA^{Trp}$ §§ | C35 | $tRNA^{Gln}$ | (100) |
| *E.coli* $tRNA^{Val}$ | U34,A35,C36<br>U34,A35,C36,A3:U70<br>U34,A35,C36,G3:C70 | $tRNA^{Met}$<br>$tRNA^{Ala}$<br>$tRNA^{Ala}$ | (79)<br>(161)<br>(161) |

The data concern switches in systems where tRNA and synthetase are from the same organisms, with host tRNAs acquiring the identity of the donor tRNA (*in vitro* experiments, except those indicated § and §§ that correspond to *in vivo* and both *in vivo* and *in vitro* studies, respectively) . Notice in the case of the Leu→Ser and Phe→Asp switches, that architectural features were also transplanted (α and β are the regions in the D-loop 5′ and 3′ from conserved G18G19; v is the length of the variable region). (a) Yeast $tRNA^{Val}$ has A73.

**Table 4.** Strength of individual identity elements as defined *in vivo* by the frequency of amino acid incorporation in a reporter protein. A few examples

| Suppressor tRNAs (*E.coli*) | mutations | % of aa incorporation: native identity | % of aa incorporation: acquired identity | Refs. |
|---|---|---|---|---|
| $tRNA^{Ala}$ (amber) | G3•U70->A3:U70 | n.d.* | n.d.* | (15,16) |
| $tRNA^{Arg}$ (amber) | (amber anticodon) | Arg(37%) | Lys(55%) | (111) |
| $tRNA^{Arg}$ (amber) | A20->U20 | n.d. | Lys(91%) | (111) |
| $tRNA^{Arg}$ (amber) | A59->U59 | Arg(38%) | Lys(50%) | (111) |
| $tRNA^{Cys}$ (amber) | U73->A73 | Cys(0%) | Lys(63%) Tyr(20%) Gln(8%) | (87) |
| $tRNA^{Cys}$ (amber) | A31:U39->C31:G39 | Cys(90%) | Gln(10%) | (88) |
| $tRNA^{Cys}$ (amber) | G29:C41->U29:A41 | Cys(93%) | Gln(5%) | (88) |
| $tRNA^{Cys}$ (amber) | G27•U43->C27:G43 | Cys(89%) | Gln(11%) | (88) |

The amber anticodon is 5′-CUA-3′. The reporter protein is DHFR expressed in *E.coli* and the strength of the identity switch is estimated by the percentage of the amino acid incorporated at position 10 of DHFR. Mutations in *E.coli* suppressor tRNAs were aimed to analyze the importance of intrinsic residues by mutation of these residues. n.d., not detectable; *, suppression efficiency near background level.

## Aminoacylation of minihelices and partial identity sets

The L-shaped architecture of tRNA with a two-fold symmetry, allows its dissection into two components corresponding to double-stranded helical structures closed by T or anticodon loops. Acceptor branch minihelices are thus constituted by acceptor and T-stems and in many cases were shown to be charged by the homologous synthetases (167,168). Prediction of function of

**Table 5.** Strength of identity elements as defined by losses of *in vitro* kinetic aminoacylation efficiencies upon their mutation in prokaryotic (A) or eukaryotic (B) systems

| L | determinants in amino acid acceptor end | | determinants in anticodon loop | | other determinants |
|---|---|---|---|---|---|
| (x-fold) | N73 | others | anticodon | others | |
| **(A) Prokaryotic systems** | | | | | |
| <10 | Arg | Ile$^{1:69}$, Trp$^{1:72}$ Val$^{1:70,4:69}$ | Glu$^{34}$ | | |
| | Lys | Asp$^{2:71}$, Gly$^{2:71,3:70}$ Gly$^{2:71,3:70}$ | Asp$^{36}$ | | Asp$^{10}$ |
| 10-100 | Ile, Trp Tyr | Met$^{2:71,3:70}$, Gln$^{1:72,3:70}$, Glu$^{1:72,2:71}$ | Glu$^{35}$ | Ile$^{36}$, Gln$^{38}$ Glu$^{37}$ | Leu$^{9*14}$ Cys$^{13:22,15*48}$ |
| | Ala, Gly Gly | Gly$^{1:72}$, His$^{-1}$, Ser$^{3:70,4:69}$ Thr$^{1:72}$, Gly$^{1:72}$ | Gly$^{36}$, Pro$^{35}$, Thr$^{36}$ Phe$^{35,36}$, Thr$^{36}$ | Asp$^{38}$ | Ala$^{20}$, Pro$^{15*48}$ Gly$^{10:25}$ |
| 100-1000 | Leu, Met Val | Gln$^{2:71}$ | Arg$^{36}$, Ile$^{34:36}$, Leu$^{35}$ Trp$^{35}$, Tyr$^{35}$ | Gln$^{37}$ | Arg$^{20}$, Gln$^{10}$ Glu$^{17:24,13:22*46}$ Ile$^{13:23,29:41}$ |
| | Pro, Phe, Thr | Pro$^{72}$, Thr$^{1:72,3:70}$ | Pro$^{36}$ Phe$^{34}$, Thr$^{35}$ | Asp$^{38}$ | Asp$^{10}$ |
| >1000 | Cys, Gln | | Arg$^{35}$, Cys$^{34}$, Gln$^{34:36}$ Met$^{34:36}$, Val$^{35}$ | | |
| | Asp, His Asp | Ala$^{3*70}$, Thr$^{2:71}$ | Asp$^{34,35}$, Gly$^{35}$, Thr$^{35}$ Asp$^{34:36}$, Gly$^{34:36}$ | | |
| **(B) Eukaryotic systems** | | | | | |
| <10 | | | Phe$^{36}$ | | |
| 10-100 | Met | Leu$^{3:70,4:69,5:68}$ | Trp$^{35}$ | Leu$^{37}$ | Leu$^{20a}$ |
| | Phe, His[a] | Gly$^{3:70}$, Thr$^{1:72}$ | His$^{34,35}$, Asp$^{36}$, Phe$^{34}$ | Asp$^{38}$ | Asp$^{10:25}$, Phe$^{20}$ |
| 100-1000 | Val | | Leu$^{35}$, Arg$^{36}$ | Met$^{loop}$ | |
| | Asp | His$^{-1}$, Gly$^{2:72}$ | Asp$^{34,35}$, Phe$^{35}$ | | |
| >1000 | Leu Leu | | Arg$^{35}$, Ile$^{34:36}$, Met$^{34:36}$ Trp$^{34}$, Val$^{35}$ | | |
| | Gly, His[b] Phe | | Gly$^{35,36}$, Thr$^{35,36}$ Phe$^{34,35}$ | Phe$^{arm}$ | Phe$^{20}$ |

Losses of aminoacylation efficiencies (L) are defined as $L = (k/K_m)_{wild\text{-}type}/(k/K_m)_{mutant}$, with k (the rate constants) being either $k_{cat}$ or $V_{max}$. When an identity residue was mutated in its three possible versions, only the strongest loss is given. Data listed on a gray background are for tRNAs aminoacylated by class I synthetases, those on a white background for tRNAs charged by class II enzymes. Identities are indicated by the amino acid three letter code; when applicable, location of identity elements in tRNA sequences are given in superscript. (A) Shows *E.coli* systems (when underlined, *T.thermophilus* systems); (B) shows *S.cerevisiae* systems (when underlined, human systems). For references see Table 1. (B) [a]15 mM $MgCl_2$, 10 mM ATP (135); [b]10 mM $MgCl_2$, 2.5 mM ATP (138).

minisubstrates containing identity determinants was first verified in the Ala system (169) (Fig. 3). Since then, aminoacylation of many other minisubstrates (Table 7) including microhelices with short helical domains or helices closed by tetraloops instead of 7 nt canonical T loops has been observed. *Escherichia coli* minihelix$^{Ala}$ and minihelix$^{His}$ with major Ala and His identity elements are good substrates for their corresponding synthetases. In contrast, minihelix$^{Ser}$ is a poor but specific substrate for SerRS, although identity nucleotides of *E.coli* tRNA$^{Ser}$ are within the acceptor arm, and no Ser determinant is found in the anticodon domain (an anticodon binding domain is missing in SerRSs). The low activity of minihelix$^{Ser}$ is probably due to the absence of the long variable region, a particular feature of tRNA$^{Ser}$ involved in recognition by SerRS (59). The efficient serylation of human tRNA$^{Ser}$ deprived of an anticodon arm but possessing its extra arm agrees with this view (176).

Minihelices recapitulating acceptor branches of tRNAs in which the major identity elements are found in the anticodon loop should *a priori* be inactive. But such minihelices were charged, although to low levels in most cases. Addition of an anticodon helix enhances charging of minihelices derived from *E.coli* tRNA$^{Ile}$ (70) and yeast tRNA$^{Val}$ (174). Asp minihelices behave like strongly anti-cooperative mutants of tRNA$^{Asp}$ and charge well (166). In contrast to a Val anticodon hairpin that stimulates valylation of the Val minihelix (174), an Asp anticodon hairpin has no effect on minihelix aspartylation. Mini- and microhelices derived from *E.coli* tRNA$^{Ile}$ are also efficiently aminoacylated by IleRS although they contain only partial recognition sets, and the strongest Ile identity elements are within the anticodon branch (Table 5).

Mutation of identity positions in minihelices impairs aminoacylation and, thus, minihelix charging follows the same identity rules as those of canonical tRNAs. However, aminoacylation

**Table 6.** Relative contributions of $k_{cat}$ and $K_m$ in tRNA identity. A few examples

| Identity (species) | Mutation | L (x-fold) | $(k_{cat})_N$ | $(K_m)_N$ |
|---|---|---|---|---|
| mostly $k_{cat}$ effect | | | | |
| Gln (Ec)* | U35->C35 | 27800 | >10 | 0.06 |
| $Met_i$ (Ec) | A35->C35 | 140 | >10 | 0.06 |
| Arg (Sc) | C35->G35 | 4920 | >10 | 0.09 |
| Glu (Ec) | U11:A24->C11:G24 | 500 | >10 | 0.10 |
| Arg (Sc) | G36->A36 | 123 | 9.38 | 0.11 |
| Arg (Sc) | G36->C36 | 45 | 7.09 | 0.14 |
| $Met_i$ (Ec) | A73->G73 | 40 | 5.47 | 0.18 |
| Asp (Sc) | G34->C34 | 400 | 5.00 | 0.20 |
| Glu (Ec) | U34->G34 | 50 | 4.77 | 0.21 |
| Ile (Ec) | G34->C34 | 320 | 4.17 | 0.24 |
| Asp (Sc) | G35->A35 | 530 | 3.84 | 0.26 |
| Leu (Sc) | A73->C73 | 16 | 3.64 | 0.27 |
| Gln (Ec)* | G73->U73 | 1667 | 3.36 | 0.30 |
| Phe (Sc) | G34->A34 | 53 | 3.12 | 0.32 |
| Phe (Sc) | A35->U35 | 263 | 3.04 | 0.33 |
| Phe (Sc) | A73->U73 | 11 | 2.43 | 0.41 |
| Asp (Ec) | C38->G38 | 44 | 1.32 | 0.75 |
| Gln (Ec)† | U35->C35 | 350 | 1.11 | 0.90 |
| mixed $k_{cat}$ and $K_m$ effect | | | | |
| Phe (Sc) | G20a->A20a | 20 | 1.03 | 0.97 |
| Ile (Ec) | A73->U73 | 43 | 0.98 | 1.02 |
| Leu (Ec) | A73->G73 | 159 | 0.93 | 1.07 |
| Asp (Ec) | G73->U35 | 195 | 0.92 | 1.08 |
| Asp (Ec) | C36->G36 | 53 | 0.81 | 1.22 |
| Gln (Ec)† | G73->U73 | 7 | 0.79 | 1.26 |
| Asp (Ec) | C36->A36 | 92 | 0.76 | 1.31 |
| Ser (Ec) | G2:C71->C2:G71 | 17 | 0.76 | 1.31 |
| mostly $K_m$ effect | | | | |
| Glu (Ec) | Δ47->U47 | 33 | 0.68 | 1.47 |
| $Met_i$ (Ec) | U36->G36 | 55 | 0.48 | 2.07 |
| Ile (Ec) | U33->A33 | 30 | 0.46 | 2.17 |
| Ile (Ec) | A76->G76 | 36 | 0.22 | 4.56 |
| Asp (Sc) | G10•U25->A10:U25 | 33 | 0.20 | 4.87 |

(Sc), *S.cerevisiae*; (Ec), *E.coli*. L-values are defined as in Table 5. For references see Table 1. The normalized values of kinetic parameters, $(k_{cat})_N$ and $(K_m)_N$, are defined as by Frugier *et al.* (162), with $(K_m)_N = [(K_m)_{mutant}/(K_m)_{wt}] \times 1/L^{1/2}]$ and $(k_{cat})_N = [(k_{cat})_{wt}/(k_{cat})_{mutant}] \times 1/L^{1/2}]$ so that $(K_m)_N \times (k_{cat})_N = 1$. *, Aminoacylation under subsaturating conditions of Gln (104); †, with steady-state kinetic data for Gln binding and GlnRS turnover (110).

**Table 7.** Aminoacylation capacities of minimalist tRNAs. A few examples

| tRNA | plateau[a] (%) | L (x-fold) | | Refs. |
|---|---|---|---|---|
| **Cys** (Ec) | 0.3 | $220x10^3$ | | (170) |
| | 0.3 | $262x10^3$ | * | |
| **Gln** (Ec) | n.d. | $35x10^6$ | * | (171) |
| **Ile** (Ec) | n.d. | 57 [e] | | (70) |
| | | 80 [e] | * | |
| **Met** (Ec) | n.d. | $\sim 10^6$-$10^8$ | | (172) |
| **Tyr** (human) | <1 | n.d. | * | (173) |
| **Val** (Sc) | 2.5 | $\sim 6x10^5$ | | (174) |
| **Ala** (Ec) | 93 | 3-4 | | (155,169,175) |
| | 1.5 | 390 | ** | |
| **Asp** (Sc) | 35 | $9x10^3$ | | (166) |
| | 25 | $32x10^3$ | * | |
| | 4 | $77x10^3$ | ** | |
| **Gly** (Ec) | 4 | $95x10^3$ | * | (129) |
| **His** (Ec) | 58 | 142 | | (134) |
| | 89 | 500 | * | |
| **Ser** (Ec) | 13 | $1x10^6$ | | (117,119) |
| | 2 | $5x10^6$ | * | |
| **Ser** (human) | 95 [b] | 3 | | (176) |
| | 3 [c] | n.d. | | |
| | 1.3 [d] | n.d. | | |

L-values (Table 5) of minimalist tRNAs are compared with those of the full-length corresponding molecules. (Ec), *E.coli*; (Sc), *S.cerevisiae*; *, data for microhelices (7 bp and T-loop); **, data for RNA tetraloops (4 bp and a stable tetraloop); n.d., not determined.

[a]Plateau are dependent on the enzyme concentration (incomplete charging is explained by deacylation events, see 164 for a discussion).

[b]Fragmented tRNA missing the anticodon.

[c]Fragmented tRNA missing both the anticodon- and D-arms.

[d]Same result for a microhelix.

[e]Additional loss of 300–500-fold when compared with tRNA with modified residues (71).

does not necessarily require the presence of major identity nucleotides. In the Asp system, impairment is equivalent in minihelix and full-length tRNA (166), whereas there are differences in the Gln and Ser systems (119,171). While the first 3 bp of the acceptor stem contribute largely to recognition of full F1 length $tRNA^{Gln}$ by GlnRS, base pairs 2–71 and 3–70 moderately affect minihelix charging. G73 keeps its role in both contexts (171). In the Ser system, contribution of G2:C71 is consistent both in full-length tRNA and in minihelicies, whereas effect of base pairs 3–70 and 4–69 is substantial in minihelices but negligible in the tRNA. Thus, investigation of minihelices reveals 'cryptic' identity elements (119).

Minihelices expressed in *E.coli*, as shown for homologous Ala and Gly (177) and *E.coli* and yeast Asp minihelices (J.Rudinger-Thirion and R.Giegé, unpublished), affect cell growth. Inhibition in Ala and Gly minihelices is correlated with the presence of identity elements in the RNA (177).

Finally, it is noticeable that minimalist RNAs corresponding to class II synthetases are generally aminoacylated better than those of class I synthetases. This may reflect a more ancient origin of class II synthetases (see below). On the other hand, minihelices constitute models for the structural understanding of identities. So, NMR studies of microhelices derived from human $tRNA^{Leu}$ suggest a decreased stability of the G1–C72 base pair when mutating the discriminator base A73 to G73 (178). This destabilization may explain the Leu→Ser switch upon mutation of the discriminator base (75,76). Similarly, distortion of the phosphodiester backbone of Ala minihelices around the G3·U70 pair revealed by NMR, may explain their Ala identity (179,180).

## Multiple identities

Each tRNA has, by definition, a single identity. However, tRNAs with multiple aminoacylation identities have been discovered in nature and could be created by engineering. A tRNA with two identities occurs naturally in certain *Candida* species (181). In these organisms, a leucine codon is to some extent translated into serine, thanks to serine-specific tRNAs [$tRNA^{Ser(CGA)}$]. These tRNAs are aminoacylated *in vitro* and *in vivo* by SerRS but also by LeuRS. Thus, two distinct amino acids are assigned by a single 'polysemous' codon (181). Interestingly, SerRS and LeuRS belong to two different classes of synthetase.

A second family of tRNAs with multiple identities is illustrated by *E.coli* $tRNA^{Ile}$ and yeast $tRNA^{Asp}$. In both cases, the naturally occurring post-transcriptional modifications hide a second, underlying identity. A minor Ile tRNA from *E.coli* ($tRNA^{Ile}_2$) is

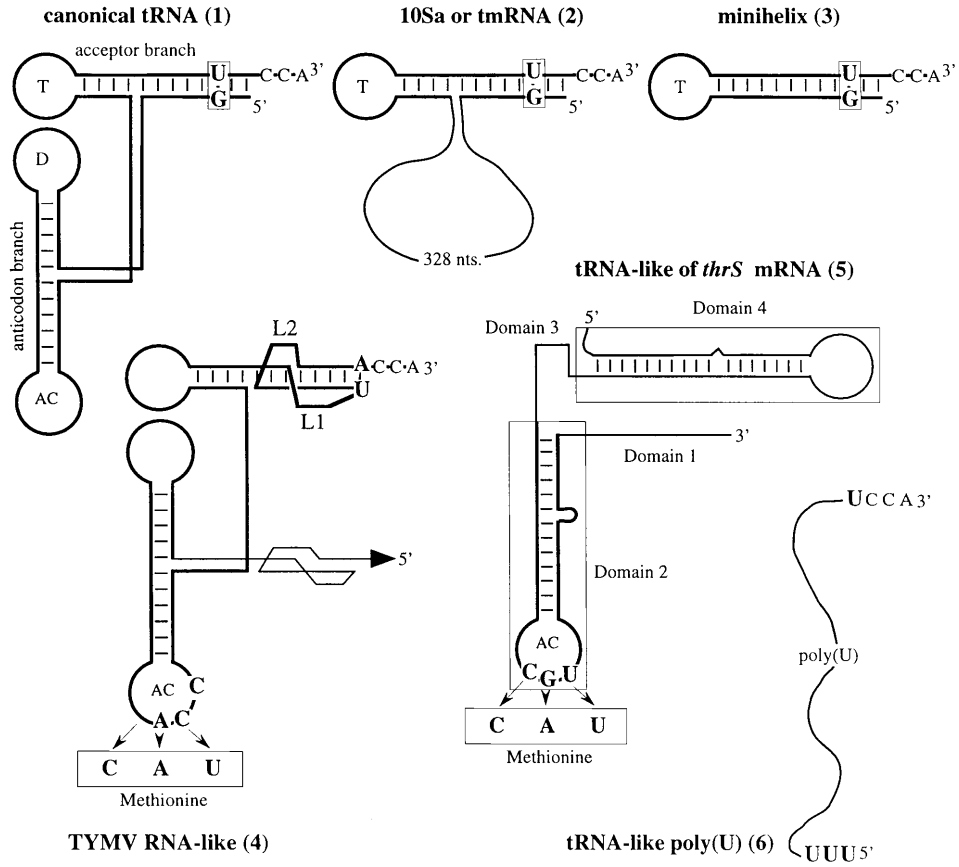


**Figure 3.** Different types of RNA scaffolds recognized by synthetases. (**1–3**) RNAs aminoacylated by AlaRS (15,16,27,169); (**4**) tRNA-like structure from TYMV aminoacylated by ValRS (22,241) and HisRS (242) and upon mutation by MetRS (236); (**5**) tRNA-like domain from the regulatory region of the *thrS* mRNA recognized by ThrRS (240,243) and upon mutation by MetRS (239); (**6**) polyU with a 3′-$CCA_{OH}$ aminoacylated by LysRS (238). The RNAs are in backbone representations with the 'tRNA' domains in bold. Identity elements for the different specificities are indicated in bold (for details see the text).

an efficient substrate for IleRS thanks to the presence of a Lysidine residue at position 34 (69). However, when this modification is removed, converting L34 to C34, the tRNA undergoes a 10-fold loss in isoleucylation efficiency and becomes a substrate for another class I enzyme, MetRS (69). A similar situation occurs in the yeast Asp system, where the *in vitro* transcript deprived of modified nucleotides has a double identity (182). It is an efficient substrate for both class II AspRS and class I ArgRS. Many other tRNAs have the potential to be recognized by non-cognate synthetases because they contain partial identity sets. This is observed for tRNAs mischarged by the same synthetase. They often have the same discriminator base and/or common anticodon residues (31).

Engineered suppressors as well as rationally designed *in vitro* transcribed chimeric tRNAs typically have several aminoacylation identities (Table 8). Suppressor tRNAs often acquire Gln or Lys identities in addition to their primary identity (46) because the corresponding identity sets include U34 and U35 present in opal (UCA) and amber (CUA) anticodons. Likewise, simultaneous introduction into yeast $tRNA^{Asp}$ of the non-overlapping Phe and Ala identity sets converts the chimeric molecule into a triple aminoacylatable substrate (150). Progressive introduction of Gln identity elements into yeast $tRNA^{Asp}$, or Asp identity elements into *E.coli* $tRNA^{Gln}$, leads to intermediate engineered tRNAs with dual specificity (142). This potential of tRNAs to display several specificities indicates a role, if not a need, of anti-determinants to restrict these possibilities (see below).

## STRUCTURAL VIEW OF IDENTITY EXPRESSION

### Contacts between tRNAs and synthetases

Six crystallographic structures of tRNA–aaRS complexes are known: the *E.coli* Gln (187), yeast Asp (188) and *T.thermophilus* Lys (189), Phe (190), Pro (191) and Ser (192) complexes. Footprinting data are available for yeast Asp (193–195), Arg (196,197), Phe (198) and Val (198,199), *E.coli* Ile (71) and Thr (200) and bean Leu (201) complexes. These data are critical to the understanding of the structural basis of identity expression. As examples, Figure 2 compares the 3D structures of the Gln and Asp complexes, representative of class I and class II systems. It emphasizes the tRNA residues in contact or close proximity with the synthetases: those are non-identity and identity elements highlighted in yellow and purple colored spheres, respectively. Contacts mainly occur at the two distal ends of the tRNA with the clear distinction that class I GlnRS interacts essentially with residues from the 5′-terminus of the tRNA and class II AspRS from the 3′-terminus. Among them, only a few are identity elements. The situation is the inverse for the anticodon stem–loop

**Table 8.** Dual and multiple specificities of tRNAs

| tRNAs | mutations | native identity | acquired identity | Refs. |
|---|---|---|---|---|
| ***In vivo* experiments** | | | | |
| $tRNA^{Ala}$ (amber)$^{(Ec)}$ | C70 | Ala(18%) | Glu(44%) Lys(29%) | (183) |
| $tRNA^{Ala}$ (amber)$^{(Ec)}$ | C70, U27•U43 | Ala(70%) | Tyr(30%) | (184) |
| $tRNA^{Asp}$ (amber)$^{(Sc)}$ | (G3)† | n.d. | Ala(74%) Lys(26%) | (185) |
| $tRNA^{Asp}$ (amber)$^{(Sc)}$ | (A38)† | n.d. | Lys(83%) Gln(17%) | (185) |
| $tRNA^{Asp}$ (amber)$^{(Sc)}$ | (ΔC56)† | n.d. | Gln(100%) | (185) |
| $tRNA^{Cys}$ (amber)$^{(Ec)}$ | (C27:G43,U29:A41, C31:G39)* | Cys(37%) | Gln(63%) | (88) |
| $tRNA^{Cys}$ (opal)$^{(Ec)}$ | $(C2:G71,G3:C70)^{Gly}$ | n.d. | Gly(93%) | (87) |
| $tRNA^{Glu}_2$ (amber)$^{(Ec)}$ | (C3:G70,C10:G25,G16)†† | no activity: "orthogonal" tRNA | | (186) |
| $tRNA^{Gly}$ (opal)$^{(Ec)}$ | $(G2:C71,C3:G70)^{Cys}$ | Gly(36%) | Cys(24%) Gln(16%) Ser(7%) | (87) |
| $tRNA^{Gly}$ (opal)$^{(Ec)}$ | (G2:C71,C3:G70 D-arm, var. region)$^{Cys}$ | n.d. | Cys (91%) | (87) |
| $tRNA^{Leu}$ (amber)$^{(Ec)}$ | $(G2:C71,A3:U70, C11:G24,C72)^{Ser}$ | Leu(99%) | n.d. | (73) |
| $tRNA^{Leu}$ (amber)$^{(Ec)}$ | $(G2:C71,A3:U70, C11:G24,C72,G73)^{Ser}$ | n.d. | Ser(92%) | (73) |
| $tRNA^{Leu}$ (amber)$^{(Ec)}$ | $(G2:C71,A3:U70, C72, G73)^{Ser}$ | Leu(38%) | Gln(39%), Ser(16%) | (73) |
| $tRNA^{Phe}$ (amber)$^{(Ec)}$ | $(C16,U17,A20, DA26,A59,U60)^{Arg}$ | n.d. | Arg(92%) | (110) |
| ***In vitro* experiments** | | | | |
| $tRNA^{Asp}$ (Sc) | removal of modified nucleotides | Asp | Arg | (182) |
| $tRNA^{Asp}$ (Sc) | transcript with Phe (Sc) and Ala (Ec) identity sets | n.d. | Ala Phe Val | (148) |
| $tRNA^{Asp}$ (Sc) | progressive introduction of Gln (Ec) identity elements | Asp | Gln | (140) |
| $tRNA^{Gln}$ (Ec) | progressive introduction of Asp (Sc) identity elements | Gln | Asp | (140) |
| $tRNA^{Ile}$ (Ec) | removal of Lysidine | Ile | Met | (69) |
| $tRNA^{Tyr}$ (Ec) | CUA amber anticodon & G3•U70 | Tyr | Ala | (90) |

(Ec), *E.coli*; (Sc), *S.cerevisiae*. The amber anticodon is 5′-CUA-3′, the opal one 5′-UCA-3′.
†The $tRNA^{Asp}$ suppressor mutants were isolated by *in vivo* selection in yeast.
††Mutations introduce 'knobs' that prevent three critical contacts with GlnRS.
n.d., not detectable.

residues, especially in the Gln system, where many residues in contact with the synthetase are identity elements.

Direct recognition of identity determinants by synthetases, as first shown in the Gln and Asp complexes, is a general theme. It is suggested in other systems by footprinting (71,193–197) and shown more directly by crystallography for anticodon identity residues contacting the synthetases in the Lys (189), Phe (190) and Pro (191) complexes. So, G35 and G36, the Pro determinants in *E.coli* (126,127) conserved in all $tRNA^{Pro}$ species, are contacted by thermophilic ProRS (191). Most contacts involve H-bonds between identity nucleotides on tRNA and identity amino acids on the synthetases, as seen in the Asp and Gln systems (165,187). These contacts are needed for activity since the decreased activity of $tRNA^{Asp}$ variants mutated at identity positions is roughly proportional to the number of H-bonds lost (164). Beside direct recognition modes, synthetases can recognize structural features in tRNA, like in the Ser complex from *T.thermophilus* where SerRS recognizes the tRNA shape through backbone interactions and without any contact with the anticodon loop (192). Recognition of specific architectural features likely accounts for the identity of many mitochondrial tRNAs with bizarre structures (202).

It is possible that identity elements act indirectly in promoting specific RNA conformations that are recognized. This could explain why mutation of G10·U25 in $tRNA^{Asp}$, which is not in direct contact with AspRS (165) leads to a $K_m$-dependent decrease in activity (141), probably because of a perturbed (N10–N25)-45 triple interaction in the mutants. The situation is more complex for Ala identity and recognition of the G3·U70 wobble pair is not definitively understood. Some data support the view of a direct recognition of this pair by AlaRS (203–206) while other data are better interpreted in the light of an indirect involvement (155,183,207,208) where the synthetase recognizes an RNA conformation induced by the wobble pair. A more definitive answer awaits crystallographic data.

The interaction mode of tRNA on multimeric synthetases has been questioned. A combined structural and genetic approach proved $tRNA^{Tyr}$ interacts with both subunits of TyRS from *Bacillus stearothermophilus*. Docking suggests that the acceptor arm interacts with the N-terminal domain of one TyrRS subunit and the anticodon arm with the disordered C-terminal domain of the other subunit (209). Similarly, crystallography shows cross-contacts of $tRNA^{Pro}$ and $tRNA^{Ser}$ over the two subunits of *T.thermophilus* ProRS (191) and SerRS (192). In the case of tetrameric PheRS, the tRNA binds across the four subunits (190). The situation is quite different in the Asp system, where each $tRNA^{Asp}$ binds to one subunit of AspRS with the exception of one H-bond between a phosphate oxygen of U1 and a lysine residue (K293) from the other subunit (165).

## Conformational changes in complexed tRNAs

Interaction with synthetases modifies the conformation of tRNA (210). This was demonstrated directly in the Gln and Asp systems by crystallography (187,188). In $tRNA^{Asp}$ the prominent deformation occurs in the anticodon loop; in contrast, in $tRNA^{Gln}$ it concerns the acceptor end with the $–CCA_{OH}$ terminus kinked and the first base pair of the stem disrupted (Fig. 2). The kink of the free $–N73CCA_{OH}$ in class I systems seems to be a necessity for geometrical reasons. This is not the case for disruption of the first base pair which is maintained in complexed $tRNA^{Val}$ interacting with class I ValRS as shown by $^{19}F$ NMR (161).

Occurrence of conformational changes in tRNA is a common characteristic and was seen by solution analysis in the Asp (195), Arg (197), Gln (211), Met (212), Ile (71) or Val (65,213) systems. The changes are likely enzyme induced as shown for the *in vitro* transcribed $tRNA^{Asp}$. It is bent over AspRS by its variable region side and over ArgRS by the opposite D-loop side (197). The deformation of the anticodon loop of $tRNA^{Asp}$ is altered if anticodon Asp identity residues are mutated and their contacts with AspRS lost. Both contacts and conformational changes are recovered if the Asp identity set is transplanted into $tRNA^{Phe}$ (195).

## Importance of tRNA architecture

Specific architectural characteristics in tRNAs can be identity elements. This is so in the variable region of $tRNA^{Ser}$ (93,117,120,121), in the atypical G15·G48 Levitt pair in *E.coli* $tRNA^{Cys}$ (86), in the G10·U25 pair in yeast $tRNA^{Asp}$ that makes a triple interaction with G45 (141), and most probably in determinants that act by $K_m$ effects (Table 6). In the *E.coli* Cys

system the functional effect of G15·G48 is determined by the A13·A22 mismatch in the D-stem of $tRNA^{Cys}$, since its introduction in $tRNA^{Gly}$ confers Cys identity (89). Similarly, in the *E.coli* Pro system, the integrity of the G15·C48 pair is important for aminoacylation (127). The case of the *E.coli* Ala system in which a structural distortion induced by the G3·U70 pair may trigger identity is discussed above.

Optimal aminoacylation efficiency often relies on conservation of specific architectural features in the core of the tRNA (142,150,214–218). For recognition by *E.coli* AlaRS (150) or GlnRS (142) and yeast PheRS (214), variable region and D-loop length is important. In the *E.coli* Pro system a relationship between ribose of U8 and G46 makes a contribution to aminoacylation (215). In yeast initiator $tRNA^{Met}$, the interplay between D- and T-loops is crucial. Interestingly, mutation of A20 and A60 into U20 and G60 abolishes methionylation (83) but not binding to MetRS (217). This is consistent with the increased stability of the mutant and with the idea that the inactive molecule is not able to adopt the correct conformation required at the transition state of the reaction.

On the other hand, synthetases can tolerate architectural variations in their tRNA substrates. Such variations can originate from the absence of base modifications (219,220) or by the replacement of individual (215,216) or families (217,218) of ribonucleotides by their deoxy counterparts. In most systems these variations are accompanied by moderate losses of charging efficiency. Note that ribonucleotides in the anticodon loop are necessary for binding of tRNA to yeast MetRS, as shown in competition experiments with anticodon hairpin DNA–RNA hybrids (217). Modifying tertiary interactions is another way to vary tRNA conformations. A systematic study on the effects of changing the sequence of the tertiary 15·48 Levitt pair in the framework of *E.coli* $tRNA^{Ala}$ found that all but an A15·A48 variant were functional *in vivo* (221). The A15·A48 mutation in the defective $tRNA^{Ala}$ variant corresponds to the Levitt pair found in human $tRNA^{Ala}$. Interestingly, this molecule has an altered conformation in the anticodon region, and introduction of the D-loop sequence of human $tRNA^{Ala}$ in the variants restores Ala activity and anticodon conformation (221).

More drastic architectural changes in tRNA compatible with specific aminoacylation have been described. The absence of T- or D-arms leads to mimics of mitochondrial tRNAs (222,223). Thus, in *E.coli* $tRNA^{Ile}$ (71) and yeast initiator $tRNA^{Met}$ (83), removal of the T-arm only slightly impairs aminoacylation. This capability of cytosolic synthetases to recognize mitochondrial-like tRNAs is supported by *in vivo* expression in *E.coli* of such molecules (224,225). Other peculiar architectural features (extended D-stem and unusual D- and T-loop interactions) exist in the core of selenocysteine inserting tRNAs and support recognition by SerRS (226). For *E.coli* $tRNA^{Sec}$ this leads to a 100-fold impaired serylation efficiency (227). Similar losses in aspartylation are found in yeast $tRNA^{Asp}$ variants with extended D-stems (228).

The most radical deviations from the canonical tRNA architecture are found in viral tRNA-like domains and in several other tRNA mimics (229–233). In the models of these mimics the identity elements are located at positions spatially similar to those in canonical tRNA. This allows similar interactions with the identity counterparts on the synthetases (Fig. 3). This explains the alanylation capacity of 10Sa tmRNA (27,232) and of minihelices (169). The histidinylation capacity of the viral tRNA-like domains is accounted for as well because the pseudoknot fold proximal to the acceptor end provides a position in loop L2 that mimics identity determinant N-1 in $tRNA^{His}$ (234,235). Likewise, the Val→Met identity switch in TYMV RNA is explained by a mutation in the anticodon and a loosening of the pseudoknot facilitating adaptation to MetRS (236,237). Furthermore, a chargeable polyU construct mimics a $tRNA^{Lys}$ with its Lys identity determinants U73 and UUU anticodon triplet (238). The binding of polyU and $tRNA^{Lys}$ to an isolated N-terminal anticodon binding domain of *E.coli* LysRS, as shown by NMR, is in line with these functional data (239). Thus, the same identity rules account for the aminoacylation capacity of all RNAs with the alternate scaffolds displayed in Figure 3. This also applies for the recognition of the *thr* mRNA regulatory structure (Fig. 3, panel 5) by synthetases, as shown by mutations in the anticodon loop mimic of this structure, making regulation of the messenger by MetRS rather than by ThrRS (240).

Thus, there is an apparent contradiction. Synthetases sense subtle architectural details in tRNAs but they can also recognize a wealth of different RNA frameworks. The flexibility of RNA allows mutual tRNA–aaRS adaptation and resolves this contradiction. Alternate scaffolds carrying identity signals can sustain synthetase recognition. Following this rationale, specific structural features in the core of tRNA may be considered as additional identity signals that enhance specificity (and in some cases even as major determinants, as for Cys identity). Another conclusion is that restricting flexibility of an RNA scaffold limits its adaptability to synthetases and favors specific recognitions. Conversely, increasing its plasticity decreases its specificity.

## A REFINED VIEW OF tRNA IDENTITY

### Bases, riboses and chemical groups

Crystallography has revealed the existence of contacts between chemical groups in nucleotide bases and the ribose phosphate backbone of tRNAs with amino acids of synthetases. However, crystallography does not indicate whether these contacts have direct consequences on the aminoacylation capacities of the interacting tRNAs, in other words if they are the actual identity determinants or are features contributing to binding in a neutral way. Functional studies on appropriate tRNA mutants are required to distinguish between the two possibilities. Several strategies can be employed for this aim, like the so-called ‘atomic mutagenesis’, crystallography combined with mutagenesis approaches, comparison of mutational effects for a single position, or the use of a T7 RNA polymerase mutant for the incorporation of deoxyriboses into RNA transcripts. A few examples of chemical groups identified by these methods as essential for aminoacylation are given in Table 9.

Atomic mutagenesis was pioneered to decipher the functional role of chemical groups in the G3·U70 Ala identity pair (203–206). To this end, minimalist RNA substrates of AlaRS were chemically synthesized with G3 or U70 replaced by base analogs. Experiments led to the conclusion that the exocyclic 2-$NH_2$ group of G3 is essential for alanylation. For Gln identity, full-length $tRNA^{Gln}$ variants were synthesized with G2, G3 or G10 identity residues replaced by inosine. Impairment of activity indicated the importance of the 2-$NH_2$ groups of these G residues for tRNA discrimination by GlnRS (109). Likewise, for Pro identity, the importance of the 2′-OH in ribose from U8 was highlighted (216).

**Table 9.** Chemical groups as essential elements for tRNA aminoacylation. A few examples

| Chemical group | Identity & species | nt (position) | Method | Refs. | Comments |
|---|---|---|---|---|---|
| **in Adenosine** | | | | | |
| N6 | Phe (Sc) | 73 | D | (149) | A>C>U † |
| | Ala (Ec) | 73 | D | (204) | A>C=U>G † |
| | Val (Ec) | 73 | D | (66) | A>C=U>G † |
| **in Uridine** | | | | | |
| O2 | Asp (Sc) | 35 | D | (244) | (*) U≥G>C>A † |
| O4 | Gln (Ec) | 35 | C | (108) | (*) U>>A>C † |
| **in Guanosine** | | | | | |
| N2 & O6 | Asp (Sc) | 73 | C | (244) | (*) G>U>>A≥C † |
| N2 & O6 | Ile (Ec) | 34 | D | (71) | G≥U>A≥C |
| N2 & N7 | Asp (Sc) | 34 | C | (244) | (*) C>>A † |
| N2 | Ala (Ec) | 3 | A | (203-206) | replacement of the G3•U70 pair by a 2-AA3•isoC10 pair led to an active variant ††. |
| N2 | Gln (Ec) | 2,3 &10 | A | (109) | G2, G3 or G10 replaced by inosine. |
| N2 | Ser (human) | 73 | A | (245) | replacement of G73 by 2-aminopurine or inosine. |
| **in Cytosine** | | | | | |
| O2 | Asp (Sc) | 36 | D | (244) | (*) G>U>A † |
| | Gln (Ec) | 34 | D | (108) | (*) C>>A † |
| **in ribose residues** | | | | | |
| U | Pro (Ec) | 8 | A | (216) | replacement of 2'-OH by 2'-H in U8 impairs charging, likely because contact between 2'-OH of U8 with N1 or N2 of G46 is broken. |
| U & G | Asp (Sc) | 11 & 27 | B | (218) | 2 subsets of tRNA with U or G's replaced by dU or dG have impaired acylation activities, likely because contacts between 2'-OH of U11 or G27 and AspRS lost. |

Methods: (A) atomic mutagenesis, (B) use of mutant T7 polymerase for incorporation of deoxyriboses into RNA transcripts, (C) crystallography combined with mutagenesis, (D) comparison of mutational effects for a same position.

†For a given position, indicates ranking of variants from the most (wt) to the less efficient one (details in refs 31 and 244).

††2-AA is 2-aminoadenosine and isoC is isocytidine (with N2 and O6).

*Predicted chemical groups found by crystallography.

Yeast $tRNA^{Asp}$ and $tRNA^{Met}$ transcripts with deoxyriboses were prepared with the mutant polymerase (218). These tRNAs fold in conformations close to those of natural tRNAs, but their *in vitro* activities indicate striking differential effects depending upon the nature of the substituted ribonucleotides. The strongest decrease in charging occurs for initiator $tRNA^{Met}$ with dG or dC and for $tRNA^{Asp}$ with dU or dG. In the Asp system, the impaired aminoacylation is correlated with the substitution of the ribose moieties of U11 and G27 that disrupt two H-bond contacts with AspRS. This suggests that specific 2′-OH groups in $tRNA^{Asp}$ are identity determinants (218).

Prediction of the chemical groups on identity bases that are H-bonded to synthetases were made on the basis of mutational analyses (31,72,244; Table 9). The approach is useful in the absence of crystallographic data. Its validity has been tested in the yeast Asp system and predictions were found in good agreement with crystallography (244).

Investigations on Ile systems led to the proposal that two functional groups (an NHCO structure) shared by identity nucleotides at the wobble position N34 in $tRNA^{Ile}$ species are the actual determinants recognized by yeast and *E.coli* IleRS. Interestingly, beside on G34 (at N1 and O6), these groups can also be brought by modified nucleotides (inosine, pseudouridine or lysidine) present at position 34 in the anticodon of $tRNA^{Ile}$ species (69,72). From another point of view, the role in aminoacylation of the hypermodified wybutine residue at position 37 in yeast $tRNA^{Phe}$ is striking. Although this residue is not a Phe determinant it plays a crucial role in the activation of the catalytic site of PheRS since $tRNA^{Phe}$ lacking this modification and the 3′-terminal A is unable to trigger aminoacylation of free A while the modified tRNA does (246).

**Table 10.** A few examples of anti-determinants

| Antideterminant | in $tRNA^{x}$ | against $aaRS^{y}$ | Refs. |
|---|---|---|---|
| G3•U70 | $tRNA^{Ala}$ (yeast)/II | ThrRS/II | (124) |
| U30•G40 | $tRNA^{Ile}$ (yeast)/I | GlnRS/I † | (248) |
| | | LysRS/II † | |
| U34 | $tRNA^{Ile}$ (*yeast*)/I | MetRS/I | (72) |
| L34 | $tRNA^{Ile}$ (*E.coli*)/I | MetRS/I | (69) |
| A36 | $tRNA^{Arg}$ (*E.coli*)/II | TrpRS/I* | (114) |
| G37 | $tRNA^{Ser}$ (yeast)/II | LeuRS/I* | (75) |
| $m^1G37$ | $tRNA^{Asp}$ (yeast)/II | ArgRS/I* | (181,247) |
| A73 | $tRNA^{Leu}$ (human)/I | SerRS/II* | (77) |

Data concern anti-determinants acting in homologous systems from a same organism, except (†) which correspond to *in vivo* expression in *E.coli* of a yeast $tRNA^{Ile}$ amber suppressor. Anti-determinants are ranked according to their occurrence in the tRNA sequence from 5′ to 3′. The class of the synthetases (I or II) is recalled. *, Class switches.

## Anti-determinants

Identity of tRNAs is not only dictated by the presence of sets of positive identity elements allowing recognition by cognate synthetases, but also by negative signals, the anti-determinants, which hinder interaction of a tRNA with non-cognate synthetases. The first anti-determinant was found in *E.coli* $tRNA^{Ile}$ where a Lysidine residue (a modified C) at position 34 of the anticodon hinders misaminoacylation by MetRS (69). Another modified nucleotide, $m^1G37$, plays a similar role in yeast $tRNA^{Asp}$ where it prohibits aminoacylation by ArgRS (182,247). Other examples of anti-determinants are given in Table 10. They are unmodified single or base-paired nucleotides located in any structural domain of the tRNA. So, anticodon residue A36 in *E.coli* $tRNA^{Arg}$ prevents recognition by TrpRS (114) and base pair U30·G40 in the anticodon stem of $tRNA^{Ile}$ prevents that by GlnRS and LysRS (248). Interestingly, protection of tRNAs from non-cognate synthetases is not restricted to synthetases from the same class. Examples in Table 10 show indeed that tRNAs which are specific substrates for class I synthetases contain anti-determinants against class II enzymes ($tRNA^{Leu}$/SerRS) and vice versa ($tRNA^{Asp}$/ArgRS). The Leu/Ser systems are of special interest since cross-protections are governed by nucleotides located at opposite positions in the tRNAs. Thus, A73 protects $tRNA^{Leu}$ against human SerRS (class II) whereas G37 protects $tRNA^{Ser}$ against yeast LeuRS (class I) (75,77).

Although only a few anti-determinants in tRNA are known, it is likely that their occurrence is not restricted to a few systems. We believe that each tRNA contains anti-determinants against several synthetases. In addition to well-defined nucleotides or to chemical groups, anti-determinants could be of a structural nature. Structural features may restrict a given tRNA from aminoacylation by a non-cognate synthetase. PheRS efficiently recognizes a

transcribed $tRNA^{Asp}$ with the five major Phe identity elements, only if the structural background has been changed from that of $tRNA^{Asp}$ to that of $tRNA^{Phe}$ (214). Another example is the so-called 'orthogonal' suppressor tRNA derived from *E.coli* $tRNA^{Gln}$. Appropriate mutations completely abolish recognition by GlnRS. These mutations introduce anti-determinants, in fact structural 'knobs', that prevent contacts with GlnRS (186).

### Permissive elements

Analysis of *in vitro* transcripts derived from yeast $tRNA^{Phe}$ and *E.coli* $tRNA^{Ala}$ bearing the complete Phe identity set (A73, G20 and the three anticodon nucleotides; Table 1) showed that PheRS is sensitive to additional nucleotides within the acceptor stem. Insertion of G2:C71 has dramatic negative effects in both tRNA frameworks. These effects are compensated by the insertion of the wobble G3·U70 pair, which by itself has no effect on phenylalanylation. From a mechanistic point of view the G2:C71 pair is not a 'classical' recognition element since its anti-determinant effect can be compensated by a second-site mutation (162). Thus, tRNA identity is not only the outcome of a combination of positive and negative signals forming the so-called identity sets, but is also based on the presence of non-random combinations of sequences elsewhere in tRNA. These sequences are 'permissive elements' and were retained by evolution so that they do not hinder aminoacylation. It is likely that no nucleotide within a tRNA is of random nature but has been selected so that a tRNA can fulfill all its functions efficiently.

### Alternate identity sets

Conceptually, each aminoacylation system was expected to have a unique set of identity elements. In other words, they should be shared by all isoaccepting tRNAs. The available data largely supports this view. However, hints suggesting that this is not completely general were obtained. Microhelix aminoacylation by a class I synthetase showed that non-conserved base pairs are required for specificity (249). In the special case of the yeast arginylation system, it has been shown that ArgRS requires two different sets of nucleotides for the specific aminoacylation of its substrates (115). Thus, arginylation of yeast $tRNA^{Arg}$ is governed by C35 and G/U36, whereas efficient arginylation of the *in vitro* transcribed version of yeast $tRNA^{Asp}$ (not protected against mischarging due to the absence of the modified bases) is based on the presence of C36 and G37 (115). Footprinting (193) and a kinetic analysis of mutants (250) are in favor of the existence of alternate mechanistic routes by which each identity set triggers the activation of the same synthetase. These unexpected facts provide new conceptual routes towards a deeper understanding of aminoacylation specificity.

### The manifold role of synthetases

Synthetases are pivotal for correct expression of tRNA identities. Physiological tRNA identities are determined by competitions among synthetases (93), reaction conditions (137,138,163) and compromises between recognition specificities and steps in the charging pathways (60,91,251–253). Furthermore, the specificity of amino acid activations and the stability of the transition state are governed by the class defining catalytic cores of the synthetases (53–57,60). On the other hand, the mutual adaptation of cognate tRNA–aaRS couples requires matching of tRNA identity determinants by protein counterparts. In contrast to the great body of data about tRNA determinants, only limited knowledge on synthetase determinants is available. No complete amino acid identity set has yet been precisely characterized. Such sets may comprise amino acids revealed by crystallography to be near to the interacting tRNAs (165,187,190).

As an illustration of the critical role of particular amino acids in the recognition of tRNAs, we give four examples. In the Gln complex, a leucine from the acceptor binding domain of GlnRS (L136) stabilizes the disruption of the U1:A72 pair in $tRNA^{Gln}$ by stacking between A72 and G2. Substituting this leucine by amino acids that would favor or disfavor stacking, modulates the discrimination potential of GlnRS against non-cognate tRNAs. Remarkably, the evolutionary solution retained has optimized both cognate tRNA recognition and discrimination against non-cognate tRNAs (253). In the Asp system, a stacking interaction of a phenylalanine (F127) between U35 and C36 in $tRNA^{Asp}$ likewise stabilizes the conformational change of the anticodon loop and thus favors matching of the anticodon identity nucleotides on AspRS (165). It is notable that this residue, as well as two other amino acids recognizing the anticodon (R119 and Q138), are conserved in the AspRS, AsnRS, LysRS class II subgroup. While these stacking interactions in the Gln and Asp systems act indirectly in facilitating other interactions, in the *E.coli* Ile and Met systems a single amino acid, namely R734 in IleRS and W461 in MetRS, is sufficient to provide recognition specificity. Indeed, the R↔W swap within homologous helix loop peptides from the anticodon binding domain of the two structurally related class I synthetases switch tRNA recognition (254). This switch is the converse of that between $tRNA^{Ile}$ and $tRNA^{Met}$ when swapping the $(G/LAU)^{Ile}$ and $(CAU)^{Met}$ anticodons (69).

Architectural motifs in synthetases are essential for tRNA recognition. One has been identified in TyrRS within its class I characteristic CP1 (connective peptide 1) sequence (173). As in tRNAs, anti-determinants exist on the surface of synthetases to repel non-cognate tRNAs (38). Only a few have been identified, such as two acidic amino acids in *E.coli* MetRS that prevent binding on non-cognate tRNA anticodons (255). Negative determinants for rejection of non-cognate tRNAs were also found in bacterial TyrRSs (209,256).

## CONSERVATION AND DIVERGENCE IN EVOLUTION

The following is based on theoretical considerations and the principle of an early molecular evolution of tRNA aminoacylation systems (167,237,257–261). Thus, contemporary tRNAs and synthetases would be derived from simplified versions restricted to the minimal structural elements needed for function. The two domains of the L-shaped tRNA would have arisen independently, with the acceptor branch appearing first. In a later stage in history, the catalytic cores of synthetases emerged independently in their class I and class II versions. We conjecture that class II cores had a primordial importance, still reflected in their modern progeny. The core is best at aminoacylating minimalist tRNAs and for two of its members (SerRS and AlaRS) does not recognize anticodon in full tRNA. Co-evolution of catalytic cores of synthetases and accepting RNA hairpins led to an operational RNA code that associated specific amino acids with RNA hairpin structures (167). Much experimental evidence supports this scenario, in particular functionality of minimalist tRNAs and conservation in

**Table 11.** Conservation of identity elements in evolution

| tRNA | | conserved identity nucleotides [a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | aa acceptor stem | | anticodon arm | | | others |
| identity & | N73* | N73 | others | anticodon | | others | |
| Val | A | A | | | A35 | | |
| Ile | A | | | | A35 | A36 | |
| Leu | A | A | | | | | |
| Met | A | A | | C34 | A35 | U36 | |
| Cys | U | U | | | | | |
| Tyr | A | A | | | U35 | | |
| Trp | G/A | | | C34 | C35 | | |
| Glu | R | | | | | | |
| Gln | R/U | | | | | | |
| Arg | R/n.c. | | | | C35 | C/U | |
| Ser | G [i] | G | | | | | |
| Thr | A/n.c. | | G1:C72 | | G35 | U36 | |
| Pro | A/C | | | | | | |
| Gly | U/A | U/A [ii] | C2:G71, G3:C70 | | C35 | C36 | |
| His | C/A | | G-1 | | | | |
| Asp | G | G | | G34 | U35 | C36 | C38 G10 |
| Lys | A/n.c. | | | | | | |
| Asn | G/n.c. | | | | | | |
| Phe | A | A | | G34 | A35 | A36 | |
| Ala | A | | G3•U70 | | | | |

[a]Experimental data are from Table 1 and concern conservations between *E.coli* (a representative of prokaryotes) and yeast, often human (the representatives of eukaryotes). Identities are displayed according to class and subclass grouping of synthetases. The dark gray shading indicates that evolutionary comparison is not possible since data from only prokaryotes are available. N73*, conserved or non-conserved (n.c.) residues in position 73 of the tRNA; (/), prokaryote/eukaryote comparison.
[i]Exception in *Candida cylindra* which has U as the discriminator base (181).
[ii]U is conserved within prokaryotic species whereas A occurs in yeast and mammalian tRNAs (48).

evolution of catalytic cores of synthetases, e.g. in AlaRSs (262), and of major identity nucleotides in acceptor branches of tRNA (Table 11).

The anticodon domain of tRNA and the additional domains of synthetases appeared later in evolution. Anticodon domains brought the link between the RNA operational code, and the correlated tRNA recognition by synthetases, with the anticodon-dependent recognition by mRNA. The additional synthetase domains introduced the source of structural diversity that led to the present idiosyncrasies in the expression of tRNA identities. As a result, for several identities (e.g. Gly, Ile, Met) the strength of identity elements in the anticodon region of tRNA overtook that of elements in the acceptor branch (Table 5).

Additional evolutionary events led to idiosyncrasies within individual identities in different species. They were often of divergent nature, but convergence also occurred. Convergent processes may account for the intriguing discovery of the first class-switch of a synthetase, namely that of an archaeal LysRS that is class I-like instead of class II (263) as well as for the origin of tRNA mimics (233). Divergent evolution may explain species differences in the functional expression of the same identity. Such differences were quantitated for Met identity in *E.coli* and yeast (80,82) and Asp identity in *E.coli*, *T.thermophilus* and yeast (143). Functional idiosyncrasies became so important for certain identities that species barriers to tRNA aminoacylation occurred, especially between prokaryotes and eukaryotes (96,264–267). For example, GlyRSs from *E.coli* and human cannot cross-charge human and *E.coli* $tRNA^{Gly}$ (265). Species barriers have been correlated with non-conservation of the discriminator nucleotide in $tRNA^{Gly}$ (265,267) or of the first base pair in the acceptor stem of $tRNA^{Tyr}$ which is G1:C72 in prokaryotes and C1:G72 in eukaryotes (96). They are compensated by changes either in tRNAs (97,148,268) or synthetases (173), probably in domains at the interface with tRNA. In line with this view is the transplantation of a 39 amino acid peptide from human to *E.coli* TyrRS that enables the bacterial enzyme to aminoacylate eukaryotic yeast $tRNA^{Tyr}$ (173). Similarly, discriminator N73 residue is A in prokaryotes and often G in eukaryotes, in the Lys identity set. Human LysRS aminoacylation is relatively insensitive to the nature of N73, a fact accounted by the peculiar sequence of 'motif 2' in the human synthetase that can accommodate degeneracy at N73 (269). Another notable example is *E.coli* $tRNA^{Tyr}$ that is a Leu acceptor in *S.cerevisiae* (270). Its identity switch is correlated with the structural similarity of the *E.coli* tRNA with yeast $tRNA^{Leu}$, both with a large variable region, while in yeast and other eukaryotes $tRNA^{Tyr}$ has a small variable region (48). Finally, domains not directly involved in the aminoacylation function of synthetases, like the N-terminal sequences that tag eukaryotic synthetases (52), may be essential to rescue prokaryotic enzymes inactive for charging of eukaryotic tRNAs. This possibility has been demonstrated in Glu systems where tagging of *E.coli* GluRS provides eukaryotic functionality to the bacterial enzyme (271). In general, species idiosyncrasies in identity are restricted to subtle sequence features in tRNAs correlated to evolutionary variations in synthetases.

From what precedes, it is likely that conservation of many major identity nucleotides reflects a common evolutionary origin (Table 11). But what about the origin of the different isoaccepting families of tRNAs? Do they each originate from a common ancestor or are other mechanisms possible? Recent genetic experiments by Saks *et al.* (272) cast doubt on the paradigm of a common ancestor. According to their 'tRNA gene recruitment' model, a tRNA gene can be recruited from one isoaccepting group to another by a point mutation in its anticodon sequence that leads to an identity switch accompanied by a change in codon recognition. The model was tested by the recruitment of a $tRNA^{Arg}$ gene that codes for a tRNA in which the Arg anticodon (UCU) is changed into a Thr anticodon (UGU) and replaces an essential $tRNA^{Thr(UGU)}$ gene that was inactivated (272). Related to such a mechanism, and based on the functional relationship in yeast of the Arg and Asp identities (115,250), is the proposal of the 'capture' of a $tRNA^{Asp}$ species by ArgRS that became a $tRNA^{Arg}$ (250).

Spectacular idiosyncrasies exist in mitochondrial systems due to the unusual structure of their tRNAs (48,222), but also to peculiarities of their genomes. Thus, a resourceful mechanism occurs in a mitochondrial gene from marsupials (273). By partial editing in a primary transcript of a $tRNA^{Asp}$ variant with Gly identity, a Gly anticodon (GCC) is changed into an Asp anticodon (GUC), so that a fraction of both $tRNA^{Asp}$ and $tRNA^{Gly}$ are produced (273). Thus, an epigenetic phenomenon compensates for the parsimony of the compact mitochondrial genome of the marsupials.

## GENERAL CONCLUSIONS

Two classes of conclusions arise from this survey on tRNA identity: first, general rules, and second, idiosyncrasies that distinguish individual or groups of identities. We list those we consider the most representative.

### (i) General rules

Similar principles govern tRNA–synthetase recognition, namely that RNA scaffolds carry a limited number of signals (the determinants) that confer recognition specificities.

Most identities rely on determinants present at both extremities of the tRNA (exceptions are the Ala and Ser identities that are anticodon-independent).

Primordial identity features are contained in the tRNA accepting branches (they may be hidden by more recent evolutionary changes in tRNA, but can be deciphered in peculiar systems, e.g. in minimalist tRNAs).

H-bonds often couple identity bases to individual amino acids of synthetases. In some cases, however, the same amino acid can H-bond with two adjacent identity bases (165).

Specific recognitions are accompanied by major conformational changes of the tRNA.

Identity sets determined *in vivo* correlate well with those determined *in vitro*. However, *in vivo* expression of identity appears less sensitive to intrinsic changes in aminoacylation kinetic efficiency than *in vitro* expression. Activity changes of variants mutated at identity positions are better revealed in isolated systems than in the cellular context where competitions due to relative concentration levels of tRNA and synthetases modulate activities.

Anti-determinants (present on either tRNAs or synthetases) restrict expression of multiple identities. Anti-determinants can be modified nucleotides.

### (ii) Idiosyncratic features

The precise interaction modes of tRNAs with the class-defining catalytic cores of synthetases and especially recognition of anticodon binding domains (if present) display great diversity.

Differences in identity expression correlated with sequence changes in tRNAs or synthetases exist between prokaryotic and eukaryotic systems; they can be strong enough to provoke species barriers.

Usage of modified nucleotides as recognition elements occur in a few identities (Glu, Ile, Lys).

Absence of certain synthetases characterizes some organisms (274).

As a final remark, it is fair to say that a number of features are not well understood in tRNA identity and that the recent discoveries of cryptic determinants, of permissive elements and of alternate identity sets have added new intricacies to the simplified classical view. It is likely that these novel features revealed in a few systems are not isolated examples. Among others, we believe that differences in identity elements may exist within isoacceptor tRNA families (e.g. in Table 1, the sequence differences at identity positions in the yeast $tRNA^{Ile}$ and $tRNA^{Gly}$ families, but which could also imply recognition of similar chemical groups). Also, relationships between identity nucleotides and more generally between non-identity nucleotides are believed to play crucial roles in identity expression. They, as well as the nature of the amino acid identity determinants on the synthetases, remain to be deciphered. The complexity in the tRNA–synthetase interrelations was well illustrated in a recent work aimed to engineer, starting from the *E.coli* Gln system, a synthetase that would charge an unnatural amino acid on an 'orthogonal' tRNA that has lost its ability to be recognized by natural synthetases (275). As noted in the accompanying commentary (276), the difficulty in finding a novel 21st tRNA–synthetase pair lies in the multifactorial nature of the RNA–protein interactions that concern not only specificity elements but also parts in tRNA and synthetase not involved in aminoacylation. It can be anticipated that such specificity manipulations will provide new openings in tRNA research. As in the above example, engineering can be based on structural knowledge combined with advanced RNA sequence analysis (277,278), but exploration of the complexity of aminoacylation systems will also benefit from combinatorial approaches in both the RNA (279) and protein (280) worlds.

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## REFERENCES

1 Crick,F.H.C. (1955) In Chargaff,E. and Davidson,J.N. (eds), *The Nucleic Acids.* Academic Press, New York, Vol. 3, p. 349.
2 Hoagland,M. (1996) *Trends Biochem. Sci.*, **21**, 77–80.
3 Rich,A. (1962) In Kasha,M. and Pullman,B. (eds), *Horizons in Biochemistry.* Academic Press, New York, pp. 103–126.
4 Lengyel,P. (1966) *J. Gen. Physiol.*, **49**, 305–330.
5 Matthaei,H., Kleinkauf,H., Heller,G., Voigt,H.-P. and Matthaei,H. (1965) In Brink,R.A. (ed.), *Proceedings of the Mendel Centenial Symposium.* The University of Wisconsin Press, Madison, pp. 105–145.
6 Chambers,R.W. (1971) *Prog. Nucleic Acid Res. Mol. Biol.*, **11**, 489–525.
7 Ebel,J.-P., Giegé,R., Bonnet,J., Kern,D., Befort,N., Bollack,C., Fasiolo,F., Gangloff,J. and Dirheimer,G. (1973) *Biochimie*, **55**, 547–557.
8 Shimura,Y. and Ozeki,H. (1973) *Adv. Biophys.*, **4**, 191–226.
9 Crothers,D.M., Seno,T. and Söll,D.G. (1972) *Proc. Natl Acad. Sci. USA*, **69**, 3063–3067.
10 Zachau,H.G. (1969) *Angew. Chem. Internat. Edit.*, **8**, 711–727.
11 Bhargava,P.M., Pallaiah,T. and Premkumar,E. (1970) *J. Theor. Biol.*, **29**, 447–469.
12 de Duve,C. (1988) *Nature*, **333**, 117–118.
13 Möller,W. and Janssen,G.M.C. (1992) *J. Mol. Evol.*, **34**, 471–477.
14 Rodin,S.N. and Ohno,S. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 5183–5188.
15 Hou,Y.-M. and Schimmel,P. (1988) *Nature*, **333**, 140–145.
16 McClain,W.H. and Foss,K. (1988) *Science*, **240**, 793–796.
17 Kisselev,L. (1985) *Prog. Nucleic Acid Res. Mol. Biol.*, **32**, 237–266.
18 Lam,S.S.M. and Schimmel,P.R. (1975) *Biochemistry*, **14**, 2775–2780.
19 Krauss,G., Riesner,D. and Maass,G. (1976) *Eur. J. Biochem.*, **68**, 81–93.
20 Schimmel,P., Söll,D. and Abelson,J.N. (eds) (1979) *Transfer RNA: Structure, Properties and Recognition.* Cold Spring Harbor Laboratory.
21 Rogers,M.J. and Söll,D. (1990) *Prog. Nucleic Acid Res. Mol. Biol.*, **39**, 185–208.
22 Pinck,M., Yot,P., Chapeville,F. and Duranton,H. (1970) *Nature*, **226**, 954–956.
23 Florentz,C. and Giegé,R. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society for Microbiology, Washington, DC, pp. 141–163.
24 Ames,B.N., Tsang,T.H., Buck,M. and Christman,M.F. (1983) *Proc. Natl Acad. Sci. USA*, **80**, 5240–5242.
25 Springer,M., Graffe,M., Dondon,J. and Grunberg-Manago,M. (1989) *EMBO J.*, **8**, 2417–2424.
26 Dardel,F., Panvert,M. and Fayat,G. (1990) *Mol. Gen. Genet.*, **223**, 121–133.
27 Komine,Y., Kitabatake,M., Yokogawa,T. and Nishikawa,K. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 9223–9277.

28 Yarus,M. (1969) *Annu. Rev. Biochem.*, **38**, 841–880.
29 Nishimura,S. (1974) In Burton,K. (ed.), *MTP International Review of Science, Biochemistry Series One, Biochemistry of Nucleic Acids*, Butterworth, London and University Park Press, Baltimore, Vol. 6, pp. 289–322.
30 Schimmel,P. and Söll,D. (1979) *Annu. Rev. Biochem.*, **48**, 601–648.
31 Giegé,R., Puglisi,J.D. and Florentz,C. (1993) *Prog. Nucleic Acid Res. Mol. Biol.*, **45**, 129–206.
32 Dreher,T.W., Bujarski,J.J. and Hall,T.C. (1984) *Nature*, **311**, 171–175.
33 Lowary,P., Sampson,J., Milligan,J., Groebe,D. and Uhlenbeck,O.C. (1986) In Van Knippenberg,P.H. and Hilbers,C.W. (eds), *Structure and Dynamics of RNA*. Plenum Press, New York and London, Vol. 110, pp. 69–76.
34 Sampson,J.R. and Uhlenbeck,O.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 1033–1037.
35 Normanly,J., Kleina,L.G., Masson,J.M., Abelson,J. and Miller,J.H. (1990) *J. Mol. Biol.*, **213**, 719–726.
36 Chattapadhyay,H., Pelka,H. and Schulman,L.H. (1990) *Biochemistry*, **29**, 4263–4268.
37 Varshney,U. and RajBhandary,U.L. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 1586–1590.
38 Normanly,J. and Abelson,J. (1989) *Annu. Rev. Biochem.*, **58**, 1029–1049.
39 Yarus,M. (1988) *Cell*, **55**, 739–741.
40 Schulman,L.H. (1991) *Prog. Nucleic Acid Res. Mol. Biol.*, **41**, 23–87.
41 Söll,D. (1991) *Experientia*, **46**, 1089–1096.
42 McClain,W.H. (1993) *FASEB J.*, **7**, 72–78.
43 McClain,W.H. (1993) *J. Mol. Biol.*, **234**, 257–280.
44 Saks,M.E., Sampson,J.R. and Abelson,J.N. (1994) *Science*, **263**, 191–197.
45 McClain,W.H. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society for Microbiology, Washington, DC, pp. 335–347.
46 Pallanck,L., Pak,M. and Schulman,L.H. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society of Microbiology, Washington, DC, pp. 371–394.
47 Hou,Y.-M. (1997) *Chem. Biol.*, **4**, 93–96.
48 Sprinzl,M., Horn,C., Brown,M., Ioudovitch,A. and Steinberg,S. (1998) *Nucleic Acids Res.*, **26**, 148–153.
49 Dirheimer,G., Keith,G., Dumas,P. and Westhof,E. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society for Microbiology, Washington, DC, pp. 93–126.
50 Auffinger,P. and Westhof,E. (1998) In Grosjean,H. and Benne,H.G.R. (eds), *Modification and Editing of tRNA*. American Society for Microbiology, Washington, DC, pp. 569–576.
51 Lapointe,J. and Giegé,R. (1991) In Trachsel,H. (ed), *Translation in Eukaryotes.* CRC Press Inc. Boca Raton, FL, pp. 35–69.
52 Mirande,M. (1991) *Prog. Nucleic Acid Res. Mol. Biol.*, **40**, 95–142.
53 Carter,C.W.,Jr (1993) *Annu. Rev. Biochem.*, **62**, 715–748.
54 Cavarelli,J. and Moras,D. (1993) *FASEB J.*, **7**, 79–86.
55 Delarue,M. and Moras,D. (1993) *Bioessays*, **15**, 675–687.
56 Cusack,S. (1995) *Nature Struct. Biol.*, **2**, 824–831.
57 Meinnel,T., Mechulam,Y. and Blanquet,S. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society for Microbiology, Washington, DC, pp. 251–290.
58 Arnez,J.G. and Moras,D. (1997) *Q. Rev. Biophys.*, **30**, 195–240.
59 Cusack,S. (1997) *Curr. Opin. Struct. Biol.*, **7**, 881–889.
60 First,E.A. (1998) In Sinnott,M. (ed), *Comprehensive Biological Catalysis*. Academic Press, New York, Vol. 1, pp. 573–607.
61 Eriani,G., Delarue,M., Poch,O., Gangloff,J. and Moras,D. (1990) *Nature*, **347**, 203–206.
62 Cusack,S., Härtlein,M. and Leberman,R. (1991) *Nucleic Acids Res.*, **19**, 3489–3498.
63 Limbach,P.A., Crain,P.F. and McCloskey,J.A. (1994) *Nucleic Acids Res.*, **22**, 2183–2196.
64 Pallanck,L. and Schulman,L.H. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 3872–3876.
65 Chu,W.-C. and Horowitz,J. (1991) *Biochemistry*, **30**, 1655–1663.
66 Tamura,K., Himeno,H., Asahara,H., Hasegawa,T. and Shimizu,M. (1991) *Biochem. Biophys. Res. Commun.*, **177**, 619–623.
67 Kern,D., Giegé,R. and Ebel,J.-P. (1972) *Eur. J. Biochem.*, **31**, 148–155.
68 Florentz,C., Dreher,T.W., Rudinger,J. and Giegé,R. (1991) *Eur. J. Biochem.*, **195**, 229–234.
69 Muramatsu,T., Nishikawa,K., Nemoto,F., Kuchino,Y., Nishimura,S., Miyazawa,T. and Yokoyama,S. (1988) *Nature*, **336**, 179–181.
70 Nureki,O., Niimi,T., Muto,Y., Kanno,H., Kohno,T., Muramatsu,T., Kawai,G., Miyazawa,T., Giegé,R., Florentz,C. and Yokoyama,S. (1993) In Nierhaus,K., Franceschi,F., Subramanian,A.R., Erdmann,V.A. and Wittmann-Liebold,B. (eds), *The Translational Apparatus.* Plenum Press, New York, pp. 59–66.
71 Nureki,O., Niimi,T., Muramatsu,T., Kanno,H., Kohno,T., Florentz,C., Giegé,R. and Yokoyama,S. (1994) *J. Mol. Biol.*, **236**, 710–724.
72 Senger,B., Auxilien,S., Englisch,U., Cramer,F. and Fasiolo,F. (1997) *Biochemistry*, **36**, 8269–8275.
73 Normanly,J., Ollick,T. and Abelson,J. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 5680–5684.
74 Asahara,H., Himeno,H., Tamura,K., Hasegawa,T., Watanabe,K. and Shimizu,M. (1993) *J. Mol. Biol.*, **231**, 219–229.
75 Soma,A., Kumagai,R., Nishikawa,K. and Himeno,H. (1996) *J. Mol. Biol.*, **263**, 707–714.
76 Breitschopf,K. and Gross,H.J. (1994) *EMBO J.*, **13**, 3166–3169.
77 Breitschopf,K., Achsel,T., Busch,K. and Gross,H.J. (1995) *Nucleic Acids Res.*, **23**, 3633–3637.
78 Uemura,H., Imai,M., Ohtsuka,E., Ikehara,M. and Söll,D. (1982) *Nucleic Acids Res.*, **10**, 6531–6539.
79 Schulman,L.H. and Pelka,H. (1988) *Science*, **242**, 765–768.
80 Lee,C.P., Dyson,M., Mandal,N., Varshney,U., Bahramian,B. and RajBhandary,U.L. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 9262–9266.
81 Meinnel,T., Mechulam,Y., Lazennec,C., Blanquet,S. and Fayat,G. (1993) *J. Mol. Biol.*, **229**, 26–36.
82 Senger,B., Despons,L., Walter,P. and Fasiolo,F. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 10768–10771.
83 Senger,B., Aphasizhev,R., Walter,P. and Fasiolo,F. (1995) *J. Mol. Biol.*, **249**, 45–48.
84 Pallanck,L., Li,S.H. and Schulman,L.H. (1992) *J. Biol. Chem.*, **267**, 7221–7223.
85 Shimizu,M., Asahara,H., Tamura,K., Hasegawa,T. and Himeno,H. (1992) *J. Mol. Evol.*, **35**, 436–443.
86 Hou,Y.-M., Westhof,E. and Giegé,R. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 6776–6780.
87 McClain,W.H. (1993) *J. Biol. Chem.*, **268**, 19398–19402.
88 Komatsoulis,G.A. and Abelson,J. (1993) *Biochemistry*, **32**, 7435–7444.
89 Hamann,C.S. and Hou,Y.-M. (1997) *Biochemistry*, **36**, 7967–7972.
90 Hou,Y.-M., Sterner,T. and Bhalla,R. (1995) *RNA*, **1**, 707–713.
91 Hou,Y.-M. and Schimmel,P. (1989) *Biochemistry*, **28**, 4942–4947.
92 Bedouelle,H. (1990) *Biochimie*, **72**, 589–598.
93 Himeno,H., Hasegawa,T., Ueda,T., Watanabe,K. and Shimizu,M. (1990) *Nucleic Acids Res.*, **18**, 6815–6819.
94 Sherman,J.M., Rogers,K., Rogers,M.J. and Söll,D. (1992) *J. Mol. Biol.*, **228**, 1055–1062.
95 Bare,L.A. and Uhlenbeck,O.C. (1986) *Biochemistry*, **25**, 5825–5830.
96 Lee,C.P. and RajBhandary,U.L. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 11378–11382.
97 Quinn,C.L., Tao,N. and Schimmel,P. (1995) *Biochemistry*, **34**, 12489–12495.
98 Himeno,H., Hasegawa,T., Asahara,H., Tamura,K. and Shimizu,M. (1991) *Nucleic Acids Res.*, **19**, 6379–6382.
99 Pak,M., Pallanck,L. and Schulman,L.D. (1992) *Biochemistry*, **31**, 3303–3309.
100 Rogers,M.J., Adachi,T., Inokuchi,H. and Söll,D. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 3463–3467.
101 Pak,M., Willis,I.M. and Shulman,L.H. (1994) *J. Biol. Chem.*, **269**, 2277–2282.
102 Yesland,K.D. and Johnson,J.D. (1993) *Nucleic Acids Res.*, **21**, 5079–5084.
103 Xue,H., Shen,W., Giegé,R. and Wong,T.-F. (1993) *J. Biol. Chem.*, **268**, 9316–9322.
104 Sylvers,L.A., Rogers,K.C., Shimizu,M., Ohtsuka,E. and Söll,D. (1993) *Biochemistry*, **32**, 3836–3841.
105 Gregory,S.T. and Dahlberg,A.E. (1995) *FEBS Lett.*, **361**, 25–28.
106 Sekine,S., Nureki,O., Sakamoto,K., Niimi,T., Tateno,M., Go,M., Kohno,T., Brisson,A., Lapointe,J. and Yokoyama,S. (1996) *J. Mol. Biol.*, **256**, 685–700.
107 Rogers,M.J. and Söll,D. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 6627–6631.
108 Jahn,M., Rogers,M.J. and Söll,D. (1991) *Nature*, **352**, 258–260.
109 Hayase,Y., Jahn,M., Rogers,M.J., Sylvers,L.A., Koizumi,M., Inoue,H., Ohtsuka,E. and Söll,D. (1992) *EMBO J.*, **11**, 4159–4165.
110 Ibba,M., Hong,K.-W., Sherman,J.M., Sever,S. and Söll,D. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 6953–6958.
111 McClain,W.H. and Foss,K. (1988) *Science*, **241**, 1804–1807.

112 Schulman,L.H. and Pelka,H. (1989) *Science*, **246**, 1595–1597.
113 McClain,W.H., Foss,K., Jenkins,R.A. and Schneider,J. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 9260–9264.
114 Tamura,K., Himeno,H., Asahara,H., Hasegawa,T. and Shimizu,M. (1992) *Nucleic Acids Res.*, **20**, 2335–2339.
115 Sissler,M., Giegé,R. and Florentz,C. (1996) *EMBO J.*, **15**, 5069–5076.
116 Normanly,J., Ogden,R.C., Horvath,S.J. and Abelson,J. (1986) *Nature*, **321**, 213–219.
117 Sampson,J.R. and Saks,M.E. (1993) *Nucleic Acids Res.*, **21**, 4467–4475.
118 Asahara,H., Himeno,H., Tamura,K., Nameki,N., Hasegawa,T. and Shimizu,M. (1994) *J. Mol. Biol.*, **236**, 738–748.
119 Saks,M.E. and Sampson,J.R. (1996) *EMBO J.*, **15**, 2843–2849.
120 Himeno,H., Yoshida,S., Soma,A. and Nishikawa,K. (1997) *J. Mol. Biol.*, **268**, 704–711.
121 Achsel,T. and Gross,H.J. (1993) *EMBO J.*, **12**, 3333–3338.
122 Schulman,L.H. and Pelka,H. (1990) *Nucleic Acids Res.*, **18**, 285–289.
123 Hasegawa,T., Miyano,M., Himeno,H., Sano,Y., Kimura,K. and Shimizu,M. (1992) *Biochem. Biophys. Res. Commun.*, **184**, 478–484.
124 Nameki,N. (1995) *Nucleic Acids Res.*, **23**, 2831–2836.
125 Nameki,N., Asahara,H. and Hasegawa,T. (1996) *FEBS Lett.*, **396**, 201–207.
126 McClain,W.H., Schneider,J. and Gabriel,K. (1994) *Nucleic Acids Res.*, **22**, 522–529.
127 Liu,H., Peterson,R., Kessler,J. and Musier-Forsyth,K. (1995) *Nucleic Acids Res.*, **23**, 165–169.
127a. Stehlin,C., Burke,B., Liu,H., Shiba,K. and Musier-Forsyth,K. (1998) *Biochemistry*, **37**, 8605–8613.
128 McClain,W., Foss,K., Jenkins,R.A. and Schneider,J. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 6147–6151.
129 Francklyn,C., Shi,J.P. and Schimmel,P. (1992) *Science*, **255**, 1121–1125.
130 Nameki,N., Tamura,K., Asahara,H. and Hasegawa,T. (1997) *J. Mol. Biol.*, **269**, 640–648.
131 Mazauric,M.-H. (1997) PhD Thesis. Université Louis Pasteur. Strasbourg.
132 Hipps,D., Shiba,K., Henderson,B. and Schimmel,P. (1995) *Proc. Natl Acad. Sci. USA*, **92**, 5550–5552.
133 Himeno,H., Hasegawa,T., Ueda,T., Watanabe,K., Miura,K. and Shimizu,M. (1989) *Nucleic Acids Res.*, **17**, 7855–7863.
134 Francklyn,C. and Schimmel,P. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 8655–8659.
135 Yan,W. and Francklyn,C. (1994) *J. Biol. Chem.*, **269**, 10022–10027.
136 Yan,W., Augustine,J. and Francklyn,C. (1996) *Biochemistry*, **35**, 6559–6568.
137 Rudinger,J., Florentz,C. and Giegé,R. (1994) *Nucleic Acids Res.*, **22**, 5031–5037.
138 Nameki,N., Asahara,H., Shimizu,M., Okada,N. and Himeno,H. (1995) *Nucleic Acids Res.*, **23**, 389–394.
139 Hasegawa,T., Himeno,H., Ishikura,H. and Shimizu,M. (1989) *Biochem. Biophys. Res. Commun.*, **163**, 1534–1538.
140 Nameki,N., Tamura,K., Himeno,H., Asahara,H., Hasegawa,T. and Shimuzu,M. (1992) *Biochem. Biophys. Res. Commun.*, **189**, 856–862.
141 Pütz,J., Puglisi,J.D., Florentz,C. and Giegé,R. (1991) *Science*, **252**, 1696–1699.
142 Frugier,M., Söll,D., Giegé,R. and Florentz,C. (1994) *Biochemistry*, **33**, 9912–9921.
143 Becker,H.D., Giegé,R. and Kern,D. (1996) *Biochemistry*, **35**, 7447–7458.
144 Li,S., Pelka,H. and Schulman,L.H. (1993) *J. Biol. Chem.*, **268**, 18335–18339.
145 McClain,W.H. and Foss,K. (1988) *J. Mol. Biol.*, **202**, 697–709.
146 Tinkle-Peterson,E., Blank,J., Sprinzl,M. and Uhlenbeck,O.C. (1993) *EMBO J.*, **12**, 2959–2967.
147 Tinkle-Peterson,E. and Uhlenbeck,O.C. (1992) *Biochemistry*, **31**, 10380–10389.
148 Sampson,J.R., DiRenzo,A.B., Behlen,L.S. and Uhlenbeck,O.C. (1989) *Science*, **243**, 1363–1366.
149 Sampson,J.R., Behlen,L.S., DiRenzo,A.B. and Uhlenbeck,O.C. (1992) *Biochemistry*, **31**, 4164–4167.
150 Frugier,M., Florentz,C., Schimmel,P. and Giegé,R. (1993) *Biochemistry*, **32**, 14053–14061.
151 Moor,N., Nazarenko,I., Ankilova,V., Khodyreva,S. and Lavrik,O. (1992) *Biochimie*, **74**, 353–356.
152 Moor,N.A., Ankilova,V.N. and Lavrik,O.I. (1995) *Eur. J. Biochem.*, **234**, 897–902.
153 Nazarenko,T.A., Tinkle-Peterson,E., Zakharova,O.D., Lavrik,O.I. and Uhlenbeck,O.C. (1992) *Nucleic Acids Res.*, **20**, 475–478.
154 Hou,Y.-M. and Schimmel,P. (1989) *Biochemistry*, **28**, 6800–6804.
155 Shi,J.P., Francklyn,C., Hill,K. and Schimmel,P. (1990) *Biochemistry*, **29**, 3621–3626.
156 Tamura,K., Asahara,H., Himeno,H., Hasegawa,T. and Shimizu,M. (1991) *J. Mol. Recog.*, **4**, 129–132.
157 Gabriel,K., Schneider,J. and McClain,W.H. (1996) *Science*, **271**, 195–197.
158 Imura,N., Weiss,G.B. and Chambers,R.W. (1969) *Nature*, **222**, 1147–1148.
159 Carneiro,V.T., Dietrich,A., Maréchal-Drouard,L., Cosset,A., Pelletier,G. and Small,I. (1994) *Plant Mol. Biol.*, **26**, 1843–1853.
160 Massire,C., Gaspin,C. and Westhof,E. (1994) *J. Mol. Graphics*, **12**, 201–206.
161 Liu,M., Chu,W.-C., Liu,J.C.-H. and Horowitz,J. (1997) *Nucleic Acids Res.*, **25**, 4883–4890.
162 Frugier,M., Helm,M., Felden,B., Giegé,R. and Florentz,C. (1998) *J. Biol. Chem.*, **273**, 11605–11610.
163 Kholod,N.S., Pan′kova,N.V., Mayorov,S.G., Krutilina,A.I., Shlyapnikov,M.G., Kisselev,L.L. and Ksenzenko,V.N. (1997) *FEBS Lett.*, **411**, 123–127.
164 Pütz,J., Puglisi,J.D., Florentz,C. and Giegé,R. (1993) *EMBO J.*, **12**, 2949–2957.
165 Cavarelli,J., Rees,B., Ruff,M., Thierry,J.-C. and Moras,D. (1993) *Nature*, **362**, 181–184.
166 Frugier,M., Florentz,C. and Giegé,R. (1994) *EMBO J.*, **13**, 2218–2226.
167 Schimmel,P., Giegé,R., Moras,D. and Yokoyama,S. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 8763–8768.
168 Martinis,S.A. and Schimmel,P. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis and Function.* American Society for Microbiology, Washington, DC, pp. 349–370.
169 Francklyn,C. and Schimmel,P. (1989) *Nature*, **337**, 478–481.
170 Hamann,C.S. and Hou,Y.-M. (1995) *Biochemistry*, **34**, 6527–6532.
171 Wright,D.J., Martinis,S.A., Söll,D. and Schimmel,P. (1993) *Biochimie*, **75**, 1041–1049.
172 Martinis,S.A. and Schimmel,P. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 65–69.
173 Wakasugi,K., Quinn,C.L., Tao,N. and Schimmel,P. (1998) *EMBO J.*, **17**, 297–305.
174 Frugier,M., Florentz,C. and Giegé,R. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 3990–3994.
175 Shi,J.-P., Martinis,S.A. and Schimmel,P. (1992) *Biochemistry*, **31**, 4931–4936.
176 Heckl,M., Busch,K. and Gross,H. (1998) *FEBS Lett.*, **427**, 315–319.
177 Hipps,D. and Schimmel,P. (1995) *EMBO J.*, **14**, 4050–4055.
178 Metzger,A.U., Heckl,M., Willbold,D., Breitschopf,K., RajBhandary,U.L., Rösch,P. and Gross,H.J. (1997) *Nucleic Acids Res.*, **25**, 4551–4556.
179 Limmer,S., Reif,B., Ott,G., Arnold,L. and Sprinzl,M. (1996) *FEBS Lett.*, **385**, 15–20.
180 Ramos,A. and Varani,G. (1997) *Nucleic Acids Res.*, **25**, 2083–2090.
181 Suzuki,T., Ueda,T. and Watanabe,K. (1997) *EMBO J.*, **16**, 1122–1134.
182 Perret,V., Garcia,A., Grosjean,H., Ebel,J.-P., Florentz,C. and Giegé,R. (1990) *Nature*, **344**, 787–789.
183 McClain,W.H., Chen,Y.M., Foss,K. and Schneider,J. (1988) *Science*, **242**, 1681–1684.
184 Hou,Y.-M. and Schimmel,P. (1992) *Biochemistry*, **31**, 10310–10314.
185 Martin,F., Reinbolt,J., Dirheimer,G., Gangloff,J. and Eriani,G. (1996) *RNA*, **2**, 919–927.
186 Liu,D.R., Magliery,T.J. and Schultz,P.G. (1997) *Chem. Biol.*, **4**, 685–691.
187 Rould,M.A., Perona,J.J., Söll,D. and Steitz,T.A. (1989) *Science*, **246**, 1135–1142.
188 Ruff,M., Krishnaswamy,S., Boeglin,M., Poterszman,A., Mitschler,A., Podjarny,A., Rees,B., Thierry,J.-C. and Moras,D. (1991) *Science*, **252**, 1682–1689.
189 Cusack,S., Yaremchuk,A. and Tukalo,M. (1996) *EMBO J.*, **15**, 6321–6334.
190 Goldgur,Y., Mosyak,L., Reshetnikova,L., Ankilova,V., Lavrik,O., Khodyreva,S. and Safro,M. (1997) *Structure*, **5**, 59–68.
191 Cusack,S., Yaremchuk,A., Kriklivij,I. and Tukalo,M. (1997) *Structure*, **6**, 101–108.
192 Biou,V., Yaremchuk,A., Tukalo,M. and Cusack,S. (1994) *Science*, **263**, 1404–1410.
193 Romby,P., Moras,D., Bergdoll,M., Dumas,P., Vlassov,V.V., Westhof,E., Ebel,J.-P. and Giegé,R. (1985) *J. Mol. Biol.*, **184**, 455–471.
194 Garcia,A. and Giegé,R. (1992) *Biochem. Biophys. Res. Commun.*, **186**, 956–962.
195 Rudinger,J., Puglisi,J.D., Pütz,J., Schatz,D., Eckstein,F., Florentz,C. and Giegé,R. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 5882–5886.
196 Gangloff,J., Jaozara,R. and Dirheimer,G. (1983) *Eur. J. Biochem.*, **132**, 629–637.
197 Sissler,M., Eriani,G., Martin,F., Giegé,R. and Florentz,C. (1997) *Nucleic Acids Res.*, **25**, 4899–4906.
198 Vlassov,V.V., Kern,D., Romby,P., Giegé,R. and Ebel,J.-P. (1983) *Eur. J. Biochem.*, **132**, 537–544.

199 Vlassov,V.V., Kern,D., Giegé,R. and Ebel,J.-P. (1981) *FEBS Lett.*, **123**, 277–281.
200 Théobald,A., Springer,M., Grunberg-Manago,M., Ebel,J.-P. and Giegé,R. (1988) *Eur. J. Biochem.*, **175**, 511–524.
201 Dietrich,A., Romby,P., Maréchal-Drouard,L., Guillemaut,P. and Giegé,R. (1990) *Nucleic Acids Res.*, **18**, 2589–2597.
202 Ueda,T., Yotsumoto,Y., Ikeda,K. and Watanabe,K. (1992) *Nucleic Acids Res.*, **20**, 2217–2222.
203 Musier-Forsyth,K., Usman,N., Scaringe,S., Doudna,J., Green,R. and Schimmel,P. (1991) *Science*, **253**, 784–786.
204 Musier-Forsyth,K., Shi,J.-P., Henderson,B., Bald,R., Fürste,J.P., Erdmann,V.A. and Schimmel,P. (1995) *J. Am. Chem. Soc.*, **117**, 7253–7254.
205 Liu,H., Yap,L.-P. and Musier-Forsyth,K. (1996) *J. Am. Chem. Soc.*, **118**, 2523–2524.
206 Beuning,P.J., Yang,F., Schimmel,P. and Musier-Forsyth,K. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 10150–10154.
207 McClain,W.H., Gabriel,K. and Schneider,J. (1996) *RNA*, **2**, 105–109.
208 McClain,W.H., Schneider,J., Bhattacharaya,S. and Gabriel,K. (1998) *Proc. Natl Acad. Sci. USA*, **95**, 460–465.
209 Bedouelle,H., Guez-Ivanier,V. and Nageotte,R. (1993) *Biochimie*, **75**, 1099–1108.
210 Rees,B., Cavarelli,J. and Moras,D. (1996) *Biochimie*, **78**, 624–631.
211 Beresten,S, Jahn,M. and Söll,D. (1992) *Nucleic Acids Res.*, **20**, 1523–1530.
212 Yamashiro-Matsumura,S. and Kawata,M. (1981) *J. Biol. Chem.*, **256**, 9308–9312.
213 Chu,W.-C., Feiz,V., Derrick,W.B. and Horowitz,J. (1992) *J. Mol. Biol.*, **227**, 1164–1172.
214 Perret,V., Florentz,C., Puglisi,J.D. and Giegé,R. (1992) *J. Mol. Biol.*, **226**, 323–333.
215 Liu,H. and Musier-Forsyth,K. (1994) *Biochemistry*, **33**, 12708–12714.
216 Yap,L.-P. and Musier-Forsyth,K. (1995) *RNA*, **1**, 418–424.
217 Aphasizhev,R., Senger,B. and Fasiolo,F. (1997) *RNA*, **3**, 489–497.
218 Aphasizhev,R., Théobald-Dietrich,A., Kostyuk,D., Kochetkov,S.N., Kisselev,L., Giegé,R. and Fasiolo,F. (1997) *RNA*, **3**, 893–904.
219 Hall,K.B., Sampson,J.R., Uhlenbeck,O.C. and Redfield,A.G. (1989) *Biochemistry*, **28**, 5794–5801.
220 Perret,V., Garcia,A., Puglisi,J., Grosjean,H., Ebel,J.-P., Florentz,C. and Giegé,R. (1990) *Biochimie*, **72**, 735–744.
221 Hou,Y.-M., Sterner,T. and Jansen,M. (1995) *Biochemistry*, **34**, 2978–2984.
222 Wolstenholme,D.R., McFarlane,J.L., Okimoto,R., Clary,D.O. and Wahleithner,J.A. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 1324–1328.
223 Watanabe,Y., Tsurui,H., Ueda,T., Furushima,R., Takamiya,S., Kita,K., Nishikawa,K. and Watanabe,K. (1994) *J. Biol. Chem.*, **269**, 22902–22906.
224 Hayashi,I., Kawai,G. and Watanabe,K. (1997) *Nucleic Acids Res.*, **25**, 3503–3507.
225 Bourdeau,V., Steinberg,S.V., Ferbeyre,G., Ewond,R., Cermahau,N. and Cedergren,R. (1998) *Proc. Natl Acad. Sci. USA*, **95**, 1375–1380.
226 Hüttenhoffer,A. and Böck,A. (1998) In Simons,R.W. and Grunberg-Manago,M. (eds), *RNA, Structure and Function.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 603–639.
227 Baron,C. and Böck,A. (1991) *J. Biol. Chem.*, **266**, 20375–20379.
228 Puglisi,J.D., Pütz,J., Florentz,C. and Giegé,R. (1993) *Nucleic Acids Res.*, **21**, 41–49.
229 Dumas,P., Moras,D., Florentz,C., Giegé,R., Verlaan,P., Belkum,A.V. and Pleij,C.W.A. (1987) *J. Biomol. Struct. Dyn.*, **4**, 707–728.
230 Felden,B., Florentz,C., Giegé,R. and Westhof,E. (1994) *J. Mol. Biol.*, **235**, 508–531.
231 Felden,B., Florentz,C., McPherson,A. and Giegé,R. (1994) *Nucleic Acids Res.*, **22**, 2882–2886.
232 Felden,B., Himeno,H., Muto,A., McCutcheon,J.P., Atkins,J.F. and Gesteland,R.F. (1997) *RNA*, **3**, 89–103.
233 Giegé,R., Frugier,M. and Rudinger,J. (1998) *Curr. Opin. Struct. Biol.*, **8**, 286–293.
234 Rudinger,J., Felden,B., Florentz,C. and Giegé,R. (1997) *Bioorg. Med. Chem.*, **5**, 1001–1009.
235 Felden,B., Florentz,C., Westhof,E. and Giegé,R. (1998) *Biochem. Biophys. Res. Commun.*, **243**, 426–434.
236 Dreher,T.W., Tsai,C.-H. and Skuzeski,J.M. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 12212–12216.
237 Giegé,R. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 12078–12081.
238 Khvorova,A.M., Motorin,Y.A., Wolfson,A.D. and Gladilin,K.L. (1992) *FEBS Lett.*, **314**, 256–258.
239 Commans,S., Plateau,P., Blanquet,S. and Dardel,F. (1995) *J. Mol. Biol.*, **253**, 100–113.
240 Graffe,M., Dondon,J., Caillet,J., Romby,P., Ehresmann,C., Ehresmann,B. and Springer,M. (1992) *Science*, **255**, 994–996.
241 Giegé,R., Briand,J.-P., Mengual,R., Ebel,J.-P. and Hirth,L. (1978) *Eur. J. Biochem.*, **84**, 251–256.
242 Rudinger,J., Florentz,C., Dreher,T. and Giegé,R. (1992) *Nucleic Acids Res.*, **20**, 1865–1870.
243 Romby,P., Caillet,J., Ebel,C., Sacerdot,C., Graffe,M., Eyermann,F., Brunel,C., Moine,H., Ehresmann,C., Ehressman,B. and Springer,M. (1996) *EMBO J.*, **15**, 5976–5987.
244 Giegé,R. (1994) *Cahiers Imabio, CNRS*, **10**, 95–101.
245 Breitschopf,K. and Gross,H.J. (1996) *Nucleic Acids Res.*, **24**, 405–410.
246 Bacha,H., Renaud,M., Lefèvre,J.-F. and Remy,P. (1982) *Eur. J. Biochem.*, **127**, 87–95.
247 Pütz,J., Florentz,C., Benseler,F. and Giegé,R. (1994) *Nature Struct. Biol.*, **1**, 580–582.
248 Büttcher,V., Senger,B., Schumacher,S., Reinbolt,J. and Fasiolo,F. (1994) *Biochem. Biophys. Res. Commun.*, **200**, 370–377.
249 Martinis,S.A. and Schimmel,P. (1993) *J. Biol. Chem.*, **268**, 6069–6072.
250 Sissler,M., Giegé,R. and Florentz,C. (1998) *RNA*, **4**, 647–657.
251 Yarus,M. (1972) *Nature New Biol.*, **239**, 106–108.
252 Avis,J.M. and Fersht,A.R. (1993) *Biochemistry*, **32**, 5321–5326.
253 Sherman,J.M. and Söll,D. (1989) *Biochemistry*, **35**, 601–607.
254 Auld,D.S. and Schimmel,P. (1995) *Science*, **267**, 1994–1996.
255 Schmitt,E., Meinnel,T., Panvert,M., Mechulam,Y. and Blanquet,S. (1993) *J. Mol. Biol.*, **233**, 615–628.
256 Bedouelle,H. and Nageotte,R. (1995) *EMBO J.*, **14**, 2945–2950.
257 Weiner,A.M. and Maizel,N. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 7383–7387.
258 Noller,H.F. (1993) In Gesteland,R.F. and Atkins,J.F. (eds), *The RNA World.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 137–157.
259 Weiner,A.M. and Maizel,N. (1994) *Curr. Biol.*, **4**, 560–563.
260 Schimmel,P. (1993) *J. Mol. Evol.*, **40**, 531–536.
261 Schimmel,P. and Ribas de Pouplana,L. (1995) *Cell*, **81**, 983–986.
262 Shiba,K., Ripmaster,T., Suzuki,N., Nichols,R., Plotz,P., Noda,T. and Schimmel,P. (1995) *Biochemistry*, **34**, 10340–10349.
263 Ibba,M., Morgan,S., Curnow,A.W., Pridmore,D.R., Vothknecht,U.C., Gardner,W., Lin,W., Woese,C.R. and Söll,D. (1997) *Science*, **278**, 1119–1122.
264 Chow and RajBhandary (1993) *J. Biol. Chem.*, **268**, 12855–12863.
265 Shiba,K., Schimmel,P., Motegi,H. and Noda,T. (1994) *J. Biol. Chem.*, **269**, 30049–30055.
266 Agou,F., Quevillon,S., Kerjan,P., Latreille,M.-T. and Mirande,M. (1996) *Biochemistry*, **35**, 15322–15331.
267 Mazauric,M.-H., Keith,G., Logan,D., Kreutzer,R., Giegé,R. and Kern,D. (1997) *Eur. J. Biochem.*, **241**, 814–826.
268 Hipps,D., Shiba,K., Henderson,B. and Schimmel,P. (1995) *Proc. Natl Acad. Sci. USA*, **92**, 5550–5552.
269 Shiba,K., Stello,T., Motegi,H., Noda,T., Musier-Forsyth,K. and Schimmel,P. (1997) *J. Biol. Chem.*, **272**, 22809–22816.
270 Edwards,H., Trézéguet,V. and Schimmel,P. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 1153–1156.
271 Whelihan,E.F. and Schimmel,P. (1997) *EMBO J.*, **16**, 2968–2974.
272 Saks,M.E., Sampson,J.R. and Abelson,J. (1998) *Science*, **279**, 1665–1670.
273 Börner,G.V., Mörl,M., Janke,A. and Pääbo,S. (1996) *EMBO J.*, **15**, 5949–5957.
274 Ibba,M., Curnow,A.W. and Söll,D. (1997) *Trends Biochem. Sci.*, **22**, 39–42.
275 Liu,D.R., Magliery,T.J., Pastnak,M. and Schultz,P.G. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 10092–10097.
276 Schimmel,P. and Söll,D. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 10007–10009.
277 Schuster,P. (1997) *Physika D*, **107**, 351–365.
278 Sagara,J.-I., Shimizu,S., Kawabata,T., Nakamura,S., Ikeguchi,M. and Shimizu,K. (1998) *Nucleic Acids Res.*, **26**, 1974–1979.
279 Illangasekare,M., Kovalchuken,O. and Yarus,M. (1997) *J. Mol. Biol.*, **274**, 519–529.
280 Frugier,M. and Schimmel,P. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 11291–11294.