Discriminating among the discriminator bases of tRNAs

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Aminoacyl-tRNA synthetases must select their specific tRNAs from the 20 structurally similar tRNAs present in a cell. The discriminator base, at position 73 of the tRNA, is important for this selection but its effects on aminoacylation are variable depending on context. Recent structural studies provide insight into this variability.

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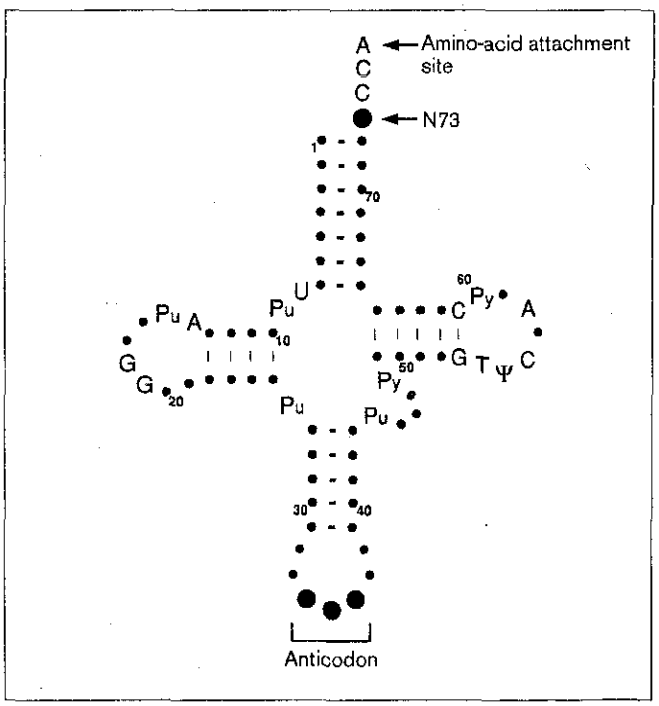
Aminoacylation of tRNAs, the attachment of amino acids to the CCA end of tRNAs (Fig. 1), is catalyzed by 20 aminoacyl-tRNA synthetases. This reaction is important for the fidelity of protein synthesis but it also poses one of the most challenging problems in molecular biology: how do the 20 synthetases discriminate between structurally similar tRNAs to achieve specific aminoacylation? One attractive possibility is that the nucleotide at position 73 (N73) adjacent to the CCA end in each tRNA might serve as a discriminator base for recognition by synthetases [1]. Recently studies of tRNA–synthetase interactions have yielded evidence that largely supports this hypothesis (Table 1); nucleotides at N73 are generally important for aminoacylation. Recent studies have also pointed out that some N73 nucleotides appear to be more important than others, however. Thus, not all N73 nucleotides are created equal and the molecular basis for this remains unknown.

Table 1 summarizes studies using tRNA variants containing a single substitution at N73. These tRNA variants are prepared by T7 RNA polymerase and their catalytic efficiency of aminoacylation (k_{cat}/K_m) is compared to that of the wild-type transcript. Most of these variants are defective for aminoacylation; however, their decrease in k_{cat}/K_m compared to that of the wild type ranges over several orders of magnitude from less than 10-fold to more than 10000-fold. The largest decrease in k_{cat}/K_m caused by a substitution of N73 in each tRNA is shown in Table 1. If this substitution causes less than a 10-fold decrease in k_{cat}/K_m ($\Delta\Delta G < 1.4$ kcal mol^{-1}), the N73 nucleotide is considered minimally important; if the decrease is 10–100-fold (1.4 kcal mol$^{-1} < \Delta\Delta G < 2.8$ kcal mol^{-1}), N73 is considered moderately important; and if it is more than 100-fold ($\Delta\Delta G > 2.8$ kcal mol^{-1}), N73 has a major role in aminoacylation. Not only do different N73 nucleotides make a different contribution to aminoacylation, but the same N73 nucleotide in different tRNAs can also have a different effect on aminoacylation (Table 1). For example, A73 has minimal significance in *Escherichia coli* $tRNA^{Lys}$, moderate significance in *E. coli* $tRNA^{Ala}$, but major significance in *E. coli* $tRNA^{Leu}$. Also, G73 of yeast $tRNA^{Asp}$ has major significance, whereas G73 of *E. coli* $tRNA^{Gln}$ has only moderate significance. For the last two examples, recent crystallographic studies of tRNA–synthetase complexes [2,3] have provided an important insight into the difference between the role of G73 of yeast $tRNA^{Asp}$ and that of E. *coli* $tRNA^{Gln}$.

The direct and the indirect mechanisms

The co-crystal structure of yeast $tRNA^{Asp}$ complexed with aspartyl tRNA synthetase indicates that G73 contributes to aminoacylation by a direct mechanism, where G73 makes direct hydrogen interactions with the synthetase [2]. In contrast, the co-crystal structure of *E. coli* $tRNA^{Gln}$

Figure 1



The cloverleaf structure of a tRNA. Variable nucleotides are indicated by small dots and the conserved and semi-conserved nucleotides are indicated by letters. The discriminator base and the anticodon nucleotides that are generally important for aminoacylation are indicated by larger dots. The numbering follows that of yeast $tRNA^{Phe}$ [22].

Table 1

The discriminator bases of tRNAs*.

Amino acid	Source of tRNA	N73	Defect of N73 tRNA variants†	Reference
Ala	*E. coli*	A	(++)	[9]
Arg	*E. coli*	A,G	+	[10]
Asn	*E. coli*	G	nd	
Asp	*E. coli*	G	+++	[11]
	Yeast	G	+++	[4]
Cys	*E. coli*	U	+++	[12]
Gln	*E. coli*	G	++	[5]
Glu	*E. coli*	G	nd	
Gly	*E. coli*	U	nd	
	Human	A	nd	
His	*E. coli*	C	+++	[6]
	Yeast	A	+++	[7]
Ile	*E. coli*	A	n.d	
Leu	*E. coli*	A	+++	[13]
Lys	*E. coli*	A	+	[10]
Met	*E. coli*	A	nd	
Phe	*E. coli*	A	+	[14]
	Yeast	A	++	[15]
Pro	*E. coli*	A	+++	[16]
Ser	*E. coli*	G	+	[17]
	Human	G	(++)	[18]
Thr	*E. coli*	A	–	[19]
Trp	*E. coli*	G	(++)	[20]
Tyr	*E. coli*	A	++	[17]
Val	*E. coli*	A	+++	[21]

*N73 is from research papers given in references or from Steinberg *et al.* [22]. †The largest defect of a substitution at N73 is represented. This defect is based on the decrease (in x-fold) of k_{cat}/K_m on aminoacylation by the substitution. Decrease of less than 10-fold is shown as '+', decrease of 10- to 100-fold is shown as '++', while decrease of greater than 100-fold is shown as '+++'. No effect on k_{cat}/K_m is shown as '–'. Not determined is shown as 'nd'. If a substitution reduces the efficiency of aminoacylation, but the value of k_{cat}/K_m is not given in the original papers, an estimate of k_{cat}/K_m is made by measuring the relative ratio of the initial rate of aminoacylation of the mutant to that of the wild type. This estimated effect is indicated by parentheses.

complexed with glutaminyl tRNA synthetase indicates that G73 acts via an indirect mechanism, where G73 does not directly interact with the synthetase but instead confers a conformational change to the acceptor end of the tRNA to facilitate aminoacylation [3].

Specifically, in the tRNA–synthetase complex of yeast $tRNA^{Asp}$ G73 extends into the active site of the enzyme where it forms hydrogen-bonding interactions with side-chains of the synthetase. It does not confer any conformational change of the acceptor stem, and therefore its contribution to aminoacylation is entirely through direct contact with the synthetase. In contrast, G73 of *E. coli* $tRNA^{Gln}$ uses its 2-amino group to make one hydrogen bond with the phosphate oxygen of the previous nucleotide, so that the backbone of G73 is folded back towards the 3′ end of the tRNA. The formation of this fold-back hairpin enables the synthetase to break open the first base pair of the acceptor stem so as to reach the second and third base pairs for specific interactions. Although the conformational change at the CCA end is necessary to stabilize the tRNA–synthetase interaction, G73 itself does not form specific hydrogen bonds with the synthetase, and therefore its contribution is entirely through an indirect mechanism.

The energetics of the direct and the indirect mechanisms can be estimated. Of the three substitutions of G73 of yeast $tRNA^{Asp}$, the G73C substitution results in the largest decrease in k_{cat}/K_m, which is 200-fold below that of the wild type [4]. This corresponds to a $\Delta\Delta G$ of 3.3 kcal mol^{-1} and represents the cost of hydrogen bonds between G73 and amino-acid sidechains of the synthetase. Of the three substitutions of G73 of *E. coli* $tRNA^{Gln}$, the G73C substitution also results in the largest decrease in k_{cat}/K_m, but the decrease here is only 14-fold and corresponds to 1.6 kcal mol^{-1} [5]. This represents the cost of one hydrogen bond between G73 and the sugar-phosphate backbone. Although detailed crystal structures of other tRNA–synthetase complexes are not yet available, it is probable that some N73 nucleotides will use the direct mechanism while others will use the indirect mechanism. The energetics of the direct mechanism should be comparable to that of G73 of yeast $tRNA^{Asp}$, whereas the energetics of the indirect mechanism may vary, depending on the complexity of the mechanism.

Is the discriminator base the major determinant of aminoacylation?

The major determinant of aminoacylation is the nucleotide in a tRNA that makes the largest thermodynamic contribution to aminoacylation. The thermodynamic contribution of N73 ($\Delta\Delta G_{73}$) is compared to those of the major and the nearest major determinants ($\Delta\Delta G_1$ and $\Delta\Delta G_2$, respectively) in several tRNAs in Table 2. The tRNAs selected for Table 2 each contain an N73 that is of at least moderate importance in aminoacylation. The data show that, while the anticodon and the discriminator bases are generally important, the discriminator base of *E. coli* $tRNA^{Cys}$, $tRNA^{His}$, $tRNA^{Leu}$, and of yeast $tRNA^{His}$ is the major determinant for aminoacylation of these tRNAs. In all cases, $\Delta\Delta G_{73}$ is equal to $\Delta\Delta G_1$ and is greater than $\Delta\Delta G_2$ by at least 1.9 kcal mol^{-1}.

Why do some discriminator bases have a dominant role in aminoacylation? One possibility is that these discriminator bases have the potential to use both the direct and the indirect mechanisms to promote the cognate tRNA–synthetase interaction. The $\Delta\Delta G$s of substitutions of U73 of *E. coli* $tRNA^{Cys}$, C73 of *E. coli* $tRNA^{His}$, and A73 of yeast $tRNA^{His}$ are 7.1 kcal mol^{-1}, 5.7 kcal mol^{-1} and 4.5 kcal mol^{-1}, respectively. All of these are greater than 3.3 kcal mol^{-1}, which is the value calculated for the direct mechanism of G73 of yeast $tRNA^{Asp}$. In fact, all of these are close to, or greater than, the $\Delta\Delta G$ of 4.9 kcal mol^{-1} that combines the direct (3.3 kcal mol^{-1}) and the indirect (1.6 kcal mol^{-1}, calculated for G73 of *E. coli* $tRNA^{Gln}$) mechanisms. This suggests that each of these discriminator bases can contribute to aminoacylation both via a direct mechanism (by providing a site of contact for the cognate synthetase) and also via an indirect mechanism (by conferring a structural feature to the tRNA to enhance the overall affinity of tRNA for the synthetase).

The direct mechanism is easy to envision. Each discriminator base will use its unique functional groups (such as O2 and O4 of U, O2 and N4 of C, N1 and N6 of A) to make specific hydrogen bonds with amino-acid sidechains of the cognate synthetase. The indirect mechanism can be proposed on the basis of sequence and structural evidence for each of the discriminator bases cited above. For example, in *E. coli* $tRNA^{His}$, C73 is paired with an extra G-1 on the 5′ end of the tRNA to form an additional base pair in the acceptor stem [6]. In yeast $tRNA^{His}$, A73 is paired with an extra G-1 to form an unusual G-1:A73 mismatch [7]. In both cases, the discriminator bases form an extra base pair that alters the structure of the acceptor end and therefore may indirectly influence the ability of the cognate synthetase to interact with the tRNA.

U73 of *E. coli* $tRNA^{Cys}$ has a propensity to fold the CCA end of the molecule towards the first nucleotide of the acceptor stem. An NMR analysis of an acceptor stem that contains the same first four base pairs and the same terminal UCCA sequence as those in *E. coli* $tRNA^{Cys}$ provides evidence for such a fold-back structure [8]. In this structure, the terminal A76 stacks with G1 and may form hydrogen bonds with U73 to stabilize a GNRA-like tetraloop structure that is commonly found in large RNAs. This fold-back structure may serve as an unusual RNA motif that is important for recognition by cysteine tRNA synthetase. In addition, the fold-back structure may allow all four nucleotides in the UCCA end to interact with the synthetase thus extending the binding interaction from one nucleotide to four nucleotides. The $\Delta\Delta G$ of the U73G substitution in *E. coli* $tRNA^{Cys}$ is 7.1 kcal mol^{-1}, which is the largest effect seen so far. It is also significantly greater than the $\Delta\Delta G$ of combined mechanisms, suggesting multiple nucleotides may be involved.

Table 2

Effect of a single nucleotide substitution on the $\Delta\Delta G$ (kcal mol^{-1}) of aminoacylation*

tRNA	Anticodon 5′–3′	N73	The largest effect	$\Delta\Delta G_1$	The second largest effect	$\Delta\Delta G_2$	The largest effect at N73	$\Delta\Delta G_{73}$	Role† of N73
Yeast Asp	GUC	G	U35A	3.8	G34C	3.7	G73C	3.3	
E. coli Gln	CUG	G	U35A	4.0	G36A	2.3	G73C	1.6	
E. coli Asp	GUC	G	U35A	5.3	G34U	5.0	G73N	2.8	
E. coli Cys	GCA	U	U73G	> 7.1	G34C	4.8	U73G	> 7.1	•
E. coli His	GUG	C	C73G	> 5.7	G-1A	2.5	C73G	> 5.7	•
Yeast His	GUG	A	A73G	4.5	G34U	2.6	A73G	4.5	•
E. coli Leu	CAG	A	A73G	3.1	G21A	0.7	A73G	3.1	•
Yeast Phe	GAA	A	A35U	3.4	G34A	2.4	A73U	1.5	
E. coli Pro	UGG	A	G72A	3.2	G36C	3.1	A73G	2.9	
E. coli Tyr	GUA	A	U35G	3.3	A73G	2.3	A73G	2.3	
E. coli Val	UAC	A	A35G	> 5.7	A73U	4.4	A73U	4.4	

*The $\Delta\Delta G$ values are calculated from the reported decrease (in x-fold) of k_{cat}/K_m of aminoacylation by a substitution according to the equation $\Delta\Delta G = -RT\ln(x)$, where R is the universal gas constant and T is the absolute temperature. In each tRNA, the largest effect of a substitution is indicated by $\Delta\Delta G_1$, the second largest effect is indicated by $\Delta\Delta G_2$, and the largest effect at N73 is indicated by $\Delta\Delta G_{73}$. The information on kcat/Km is obtained from references cited in Table 1. †The tRNAs in which the discriminator base has a dominant role in aminoacylation are indicated by large dots.

In principle, contributions to aminoacylation from the direct and indirect mechanisms should be experimentally distinguishable. For example, to determine if U73 of *E. coli* $tRNA^{Cys}$ uses a direct mechanism, tRNA variants that are defective in the direct mechanism but competent in the indirect mechanism can be created. These variants may lack U73 but retain the ability to form the fold-back structure. Conversely, to determine if U73 of *E. coli* $tRNA^{Cys}$ contributes indirectly to aminoacylation, tRNA variants that are defective in the indirect mechanism but competent in the direct mechanism can be created. These variants may retain U73 but lack the ability to form a stable fold-back structure. Both classes of mutants can be assayed for aminoacylation and their decrease in k_{cat}/K_m can be used to estimate the energetics of the direct or indirect mechanism. Additionally, both classes of mutants can be characterized for the presence or absence of the fold-back structure to provide a correlation between the structure and function of U73.

The original discriminator-base hypothesis has turned out to be partially correct, but the true mechanism of discrimination in tRNA recognition is far more complex than originally imagined — and far more interesting.

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