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Identity elements of *Saccharomyces cerevisiae* $tRNA^{His}$

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ABSTRACT

Recognition of $tRNA^{His}$ by *Saccharomyces cerevisiae* histidyl-tRNA synthetase was studied using *in vitro* transcripts. Histidine tRNA is unique in possessing an extra nucleotide, G_{-1}, at the 5′ end. Mutation studies indicate that this irregular secondary structure at the end of the acceptor stem is important for aminoacylation with histidine, while the requirement of either base of this extra base pair is smaller than that in *Escherichia coli*. The anticodon was also found to be required for histidylation. The regions involved in histidylation are essentially the same as those in *E.coli*, whereas the proportion of the contributions of the two portions distant from each other, the anticodon and the end of the acceptor stem, makes a substantial difference between the two systems.

INTRODUCTION

The fidelity of protein biosynthesis depends upon the correct aminoacylation of tRNAs by their cognate aminoacyl-tRNA synthetase. Several technical advances are settling the problem of how aminoacyl-tRNA synthetase can specifically recognize cognate tRNAs from a pool of various tRNA species sharing a similar tertiary structure (1–3). These studies have indicated that a relatively small number of nucleotides specify the amino acid acceptor identity of tRNA, which usually include the anticodon nucleotides and the discriminator base at position 73 (the base preceding the 3′ terminal CCA) as major recognition sites (4–19). In addition to these major recognition nucleotides, structural features are often recognized by cognate aminoacyl-tRNA synthetase (20–22).

Histidine tRNA possesses a conspicuous feature in its structure in that the 5′ end is one-nucleotide longer than other tRNA species (23,24). This extra nucleotide (G_{-1}) is opposite to the discriminator base, consequently comprising a one-base-pair longer acceptor stem. The processing mechanism of this unusual tRNA structure is phylogenetically divergent. In prokaryotes, chloroplasts, and plant or fungal mitochondria, irregular processing by RNase P leaves the gene-encoded G_{-1} in the mature $tRNA^{His}$ (25–31). In eukaryote cytoplasmic $tRNA^{His}$, G_{-1} is not encoded on the gene but is added post-transcriptionally by a specific tRNA guanylyl transferase (32). The exclusive occurrence of G_{-1} notwithstanding the phylogenetic divergence of maturation of $tRNA^{His}$ implies the importance of the noncanonical clover-leaf structure for some histidine-specific function, and the role in histidine-specific aminoacylation has been exemplified in the *Escherichia coli* system using *in vitro* transcripts (7). This has also been confirmed by an observation that a minihelix structure comprising only an acceptor stem and a T-arm of $tRNA^{His}$ is aminoacylable with histidine (33). In order to attribute this unique secondary structure of $tRNA^{His}$ to the histidine specific aminoacylation, recognition of $tRNA^{His}$ from various species bears further investigation. Although this irregular clover-leaf structure is phylogenetically conserved, the opposing discriminator base is divergent between prokaryotes or organelles (C_{73}) and eukaryotes (A_{73}) (23). Here, we investigate the recognition elements of $tRNA^{His}$ from a eukaryote, *Saccharomyces cerevisiae*, revealing some similarities and differences between the yeast and *E.coli* systems.

MATERIALS AND METHODS

Preparation of template DNAs and *in vitro* transcripts

Synthetic DNA oligomers carrying the T7 promoter and tRNA genes were ligated into pUC19 and transformed into *E.coli* strain JM109 (1,7,8). The template DNA sequences were confirmed by dideoxy sequencing (34). Each template DNA of the discriminator base-substituted mutant was prepared from a plasmid carrying the normal tRNA sequence and two synthetic primers by mutation using the polymerase chain reaction (35). Transcripts of the tRNA genes were prepared in a reaction mixture containing 40 mM Tris–HCl (pH 8.1), 5 mM dithiothreitol, 2 mM spermidine, 10 mM magnesium chloride, bovine serum albumin (50 μg/ml), 2.0 mM each NTP, 20 mM 5′ GMP, *Eco*T22I-digested template DNA (0.2 mg/ml), 2 U of inorganic pyrophosphatase (Sigma) and pure T7 RNA polymerase (50 μg/ml) (1,7,10). Transcripts initiated with A and C were prepared in a reaction mixture containing 20 mM 5′ AMP and 5′ CMP, respectively, instead of 5′ GMP (7). A transcript initiated with a 5′ triphos-

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phorylated guanosine was prepared in the absence of 5′ GMP and inorganic pyrophosphatase (1,7). The transcripts were purified by 20% polyacrylamide gel electrophoresis.

Aminoacylation assay

Histidyl-tRNA synthetase was partially purified from *S.cerevisiae* strain BJ926 (kindly provided by Dr Y. Ohsumi of the University of Tokyo) by column chromatographies with DEAE-Toyopearl 650 (Tosoh) and CM-Sephadex C-50 (Pharmacia). The final enzyme fraction had a specific activity of 920 U (1 U of histidyl-tRNA synthetase activity was defined as the amount of enzyme which catalyzes the incorporation of 1 nmol of histidine into histidinyl-tRNA in 10 min) per mg protein. The aminoacylation reaction was performed at 30°C in a buffer containing 60 mM Tris–HCl (pH 7.5), 10 mM magnesium chloride, 30 mM potassium chloride, 5 mM dithiothreitol, 2.5 mM ATP and 15 μM L-[U-^{14}C] histidine (12.5 GBq/mmol), with various concentrations of tRNA transcripts and *S.cerevisiae* histidyl-tRNA synthetase. The aminoacylation with *E.coli* histidyl-tRNA synthetase proceeded at 37°C, the other conditions being in accordance with aminoacylation using *S.cerevisiae* histidyl-tRNA synthetase. The initial rates of aminoacylation were determined by using six concentrations of tRNA transcripts ranging from 0.10 to 6.0 μM at a fixed concentration of histidyl-tRNA synthetase, depending on the mutant tRNA transcripts.

RESULTS AND DISCUSSION

The extra base pair, G_{-1}–A_{73}

$tRNA^{His}$ contains an extra 5′ nucleotide which can pair with the discriminator base at position 73. Himeno *et al.* have previously shown that this extra base pair, G_{-1}–C_{73}, is responsible for aminoacylation with *E.coli* histidyl-tRNA synthetase (7). We examined the mutation effects on the aminoacylation kinetics of *S.cerevisiae* $tRNA^{His}$ transcripts at this position (G_{-1}–A_{73}) with *S.cerevisiae* histidyl-tRNA synthetase (Table 1). The most pronounced effect occurred at the A_{73} to G_{73} substitution, which severely affected the V_{max}, though with little effect on K_m. In the transcript with an additional G_{-1} to A_{-1} mutation, the histidine accepting activity was also damaged by the A_{73} to G_{73} substitution. Other substitutions at this position reduced the V_{max}/K_m only 3- to 13-fold. The order of base preference was changed when G_{-1} was substituted by A_{-1}. These results raise the possibility that A_{73} has no direct interaction with histidyl-tRNA synthetase, and that other bases, particularly G_{73}, behave as obstacles to the normal positioning of the CCA end in aminoacylation. This is in an apparent contradiction to another study showing that an A to G mutation at the putative discriminator position of a tRNA-like structure corresponding to the 3′ end of the turnip yellow mosaic virus RNA does not seriously affect aminoacylation with yeast histidyl-tRNA synthetase (36). Particular impairment by G_{73} has also been observed in *E.coli* (7). This type of biased base preference appears effective for histidyl-tRNA synthetase in discriminating other tRNAs possessing G at the discriminator position, such as $tRNA^{Asp}$ and $tRNA^{Asn}$.

Removal of G_{-1} resulted in a 5-fold increase in K_m and a 170-fold decrease in V_{max}, indicating that this extra nucleotide plays a crucial role in aminoacylation with histidine. However, base substitutions at this position had much less effect. The A_{-1} and C_{-1} mutants had V_{max}/K_ms only 6- and 9-fold smaller than the wild-type transcript, respectively. A simultaneous mutation of G_{-1} to A_{-1} and A_{73} to C_{73} caused a more moderate (only 2-fold) decrease of activity. These findings suggest that histidyl-tRNA synthetase has direct interaction with the extra backbone phosphate and/or ribose but it makes no functional interaction with the base moiety of G_{-1} or that of A_{73}. The 5′ triphosphorylated transcript had a much lower activity than the normal monophosphorylated transcript (Table 1), indicating that the 5′ terminal phosphate of $tRNA^{His}$ is in proximity to histidyl-tRNA synthetase during aminoacylation. A similar effect by triphosphorylation has been observed in the *E.coli* histidine specific aminoacylation system (7).

The above results indicate that the structural irregularity at the end of the acceptor stem is crucial for specific aminoacylation with histidyl-tRNA synthetase, although the base requirement of either G_{-1} or A_{73} is much more relaxed than that in *E.coli* (7).

Acceptor stem

The three base pairs next to the extra base pair, in particular the first base pair (G_1–C_{72}), are well conserved among eukaryotic $tRNA^{His}$. Substitution of either G_1–C_{72} or G_2–C_{71} by C–G had no effects on aminoacylation, while substituting C_3–G_{70} by G–C reduced the V_{max}/K_m 3-fold. This indicates that the acceptor stem other than G_{-1}–A_{73} is not important for recognition by yeast histidyl-tRNA synthetase.

Anticodon arm

Base substitutions were made at each position of the anticodon triplet GUG of yeast $tRNA^{His}$. Substitution of either G_{34} or U_{35} by any of the other three bases resulted in a decrease of V_{max}/K_m by a factor of 10–70, while changing G_{36} caused about a 4-fold decrease (Table 1). In the vast majority of tRNA species, aminoacylation efficiency is severely impaired by mutation in the anticodon region, which usually affects both V_{max} and K_m (5,7–9,11,12,14,15,17,18,20,37). An exception is the yeast tyrosine system, in which the mutation effect is mainly attributable to K_m (38). Anticodon mutations in this study also resulted in only a weak impairment of V_{max}, suggesting that the recognition process of the anticodon is independent of the catalytic process of histidylation. The possibility that such a behaviour of the kinetic parameters by anticodon mutation is attributable to the indirect conformational effect is not completely ruled out. However, this seems unlikely to occur, since every mutation in the anticodon caused decrease of activity. The involvement of the anticodon, as well as of the lower half of the anticodon stem, in recognition by yeast histidyl-tRNA synthetase has been suggested by a footprinting study using a tRNA-like structure corresponding to the 3′ end of the turnip yellow mosaic virus RNA (39). Mutation was extended to the base pair in the anticodon stem, C_{30}–G_{40}, which is conserved within the $tRNA^{His}$ isoacceptors. This mutation resulted in only a small impairment of histidylation (Table 1).

Table 1. Kinetic parameters with *S.cerevisiae* histidyl-tRNA synthetase for the transcripts

	K_m (μM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of efficiency (x-fold)
Yeast $tRNA^{His}$ derivatives				
Wild-type	0.35	100	1	1
Extra base pair, G_{-1}–A_{73}				
G_{-1}–C_{73}	0.62	46	0.25	4
G_{-1}–U_{73}	1.6	59	0.13	8
G_{-1}–G_{73}	0.49	0.12	8.2×10^{-4}	1200
A_{-1}–A_{73}	1.2	57	0.17	6
A_{-1}–C_{73}	0.80	110	0.46	2
A_{-1}–U_{73}	0.85	19	0.078	13
A_{-1}–G_{73}	0.36	0.076	7.3×10^{-4}	1400
C_{-1}–A_{73}	0.79	27	0.12	9
C_{-1}–C_{73}	0.55	34	0.22	5
C_{-1}–U_{73}	2.7	65	0.081	12
$pppG_{-1}$–A_{73}	2.7	120	0.15	7
G_{-1}-deficient mutants				
A_{73}	1.7	0.58	1.4×10^{-3}	740
C_{73}	1.1	3.6	9.7×10^{-3}	100
U_{73}	2.7	0.71	8.8×10^{-4}	1100
Acceptor stem				
C_1–G_{72}	0.30	85	0.97	1
G_2–C_{71}	0.19	91	1.63	1
G_3–C_{70}	0.79	77	0.34	3
Anticodon				
$A_{34}U_{35}G_{36}$	1.3	15	0.040	25
$C_{34}U_{35}G_{36}$	1.8	36	0.068	15
$U_{34}U_{35}G_{36}$	4.8	20	0.014	70
$G_{34}A_{35}G_{36}$	2.7	46	0.58	17
$G_{34}G_{35}G_{36}$	2.4	49	0.70	14
$G_{34}C_{35}G_{36}$	3.6	42	0.40	25
$G_{34}U_{35}A_{36}$	1.0	78	0.26	4
$G_{34}U_{35}C_{36}$	1.2	80	0.23	4
$G_{34}U_{35}U_{36}$	1.2	96	0.28	4
Anticodon stem				
C_{30}–G_{40}	0.42	65	0.53	2
E.coli $tRNA^{His}$ derivatives				
Wild-type	1.0	25	0.085	12
G_{-1}–A_{73}	0.64	48	0.26	4
E.coli $tRNA^{Asp}$ derivatives				
Wild-type			$<1.0 \times 10^{-4}$	>10000
G_{-1}–G_{73}	3.8	0.60	5.5×10^{-4}	1800
G_{-1}–A_{73}	1.3	15	0.039	26
yeast $tRNA^{Pro}$ derivatives				
Wild-type			3.2×10^{-4}	3100
G_{-1}–C_{73}	3.1	11	0.013	78
G_{-1}–C_{73},$G_{34}U_{35}G_{36}$	0.76	8.9	0.040	25

The position of mutation is underlined. Each parameter was determined from a plot of [S] against [S]/v ([S], tRNA concentration; v, observed initial velocity of histidylation).

Transplantation of G_{-1}–A_{73} and the anticodon sequence into other tRNAs

The above results indicate the particular importance of the extra base pair G_{-1}–A_{73}, and the first and second letters of the anticodon. We made attempts to introduce these elements into other tRNAs.

The *E.coli* $tRNA^{His}$ transcript, which possesses G_{-1}, G_{34}, U_{35} and G_{36} (Fig. 1b), was a relatively good substrate for yeast histidyl-tRNA synthetase, with a V_{max}/K_m 12-fold lower than that of the yeast $tRNA^{His}$ transcript (Table 1b). An additional mutation of C_{73} to A_{73} elevated the V_{max}/K_m to 4-fold lower than that of the yeast $tRNA^{His}$ transcript.

Next, a G_{-1} was added into the *E.coli* $tRNA^{Asp}$ transcript, where the first and second letters of the anticodon are identical to those of $tRNA^{His}$ (Fig. 1c). This mutant had a faint but detectable histidine accepting activity with a V_{max}/K_m 1800-fold lower than that of the yeast $tRNA^{His}$ transcript (Table 1). As expected, an

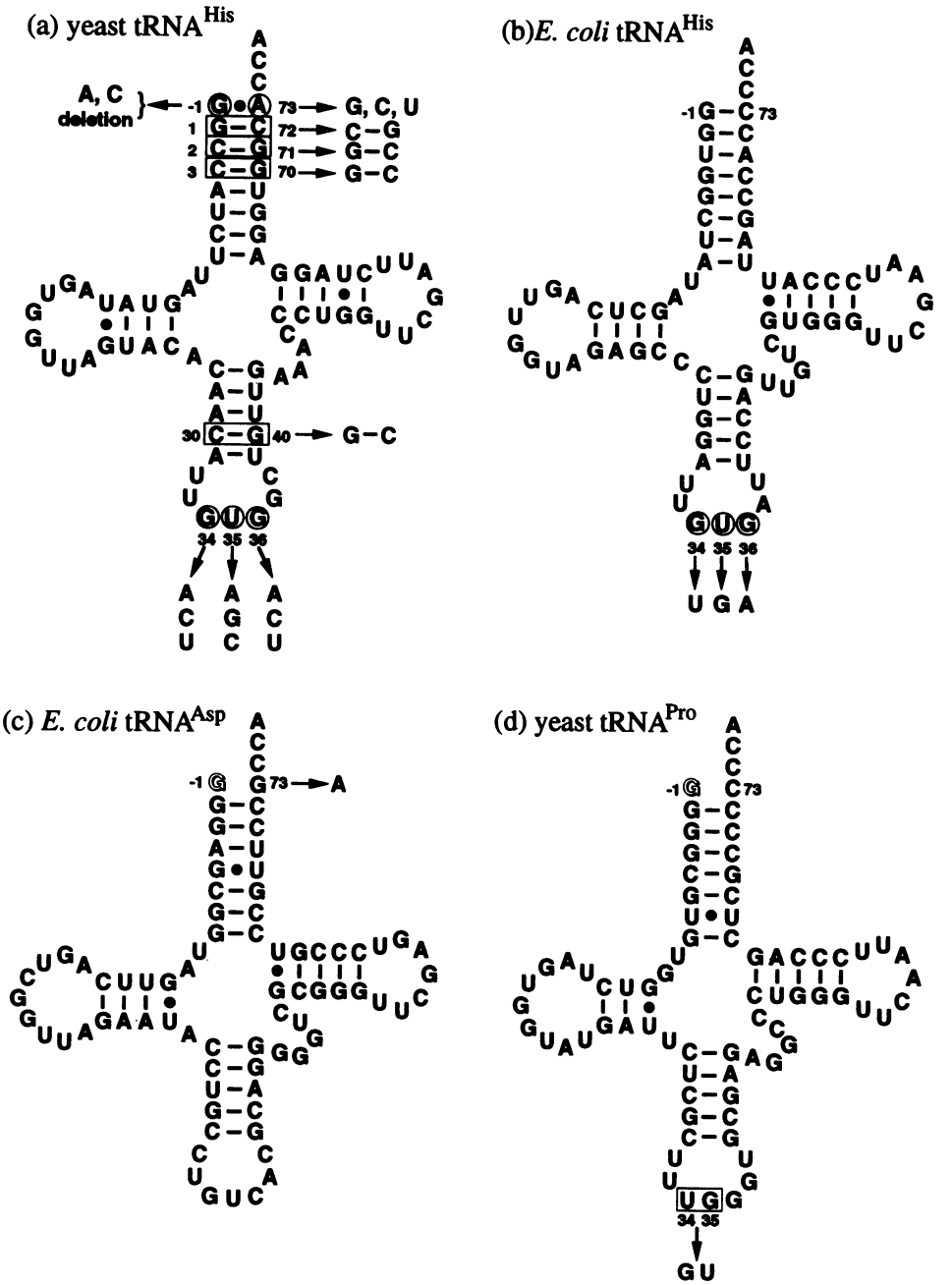

Figure 1. Cloverleaf representations of: (**a**) *S.cerevisiae* tRNA^{His}, (**b**) *E.coli* tRNA^{His}, (**c**) *E.coli* tRNA^{Asp} and (**d**) *S.cerevisiae* tRNA^{Pro}, with the base modifications omitted. Numbering is according to (23). Arrows indicate the substitutions and deletion in this study. Insertion of G_{-1} is designated as an outline. Non-Watson–Crick type of base pair is indicated by a filled circle.

additional G_{73} to A_{73} mutation elevated the activity to a 25-fold lower V_{max}/K_m.

We also transplanted the extra G_{-1} and the histidine anticodon GUG into the yeast tRNA^{Pro} transcript (Fig. 1d). This tRNA^{Pro} mutant had a histidine accepting activity with a V_{max}/K_m 25-fold lower than the tRNA^{His} transcript (Table 1).

Recognition of the anticodon with *E.coli* HisRS

As for histidyl-tRNA synthetase recognition, the system that has been best studied is that of *E.coli*, in which the extra base pair G_{-1}–C_{73} is crucial for histidine specific aminoacylation (7). In order to further assess the difference between the yeast and *E.coli* systems, we constructed *E.coli* tRNA^{His} transcripts with mutations in the anticodon (Fig. 1b). These mutations reduced the aminoacylation activity with *E.coli* histidyl-tRNA synthetase by factors of 3–7 (Table 2). This indicates that the anticodon is involved in recognition by histidyl-tRNA synthetase in *E.coli* as well, but the contribution is much lower than that in yeast. However, this kind of small loss of activity upon base substitution always leaves an alternative possibility that although synthetase does not directly interact with the base of mutation, the resulting conformational distortion affects the normal interaction. As is in

the case of the yeast system, these anticodon mutations in *E.coli* did not significantly affect the V_{max}.

Concluding remarks

The results obtained in this study show that yeast histidyl-tRNA synthetase recognition requires the irregular secondary structure at the end of the acceptor stem and the anticodon sequence. This is essentially the same as recognition by *E.coli* histidyl-tRNA synthetase. However, the contributions of the two portions makes a substantial difference between the two systems. In *E.coli*, the extra base pair of the acceptor stem is more important than the anticodon. Unlike the *E.coli* system, in yeast the contribution of the anticodon is increased while the importance of the discriminator base is decreased. The present kinetic studies also suggest that in both yeast and *E.coli* histidyl-tRNA synthetase the catalytic domain is functionally separated from the anticodon binding domain. If the latter domain had been added to the former at a late stage of evolution, the yeast system would have changed more extensively from the ancient form.

Table 2. Kinetic parameters with *E.coli* histidyl-tRNA synthetase for the transcripts

E.coli $tRNA^{His}$ derivatives	K_m (μM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of efficiency (x-fold)
Wild-type	0.46	100	1	1
$\underline{U}_{34}U_{35}G_{36}$	2.9	92	0.15	7
$G_{34}\underline{G}_{35}G_{36}$	1.2	69	0.27	4
$G_{34}U_{35}\underline{A}_{36}$	1.8	82	0.21	5

The position of mutation is underlined. Histidyl-tRNA synthetase enriched fraction from *E.coli* which had a specific activity of 14 U/mg protein was used. Each parameter was determined from a plot of [S] against [S]/v ([S], tRNA concentration; v, observed initial velocity of histidylation).

The apparent uniformity of the tRNA structure is thought to complicate the specific recognition of cognate tRNAs without error. It thus seems natural that aminoacyl-tRNA synthetase preferentially recognizes characteristic features such as the long variable arm of $tRNA^{Ser}$ (22,40,41) and the wobble G–U base pair in the acceptor stem (42,43). Probably due to their functional constraint, such characteristics are often conserved among various species (23,44). Although the unique secondary structure of $tRNA^{His}$ was conserved during evolution, the kind of discriminator base diverged. Note that C_{73} is unique to $tRNA^{His}$ in *E.coli*, but A_{73} is predominant among yeast tRNAs (23). This indicates that C_{73} in *E.coli* is much more appropriate for discrimination from noncognate tRNAs than A_{73} in yeast. It is thus quite reasonable that *E.coli* histidyl-tRNA synthetase recognizes this portion more preferably than the yeast synthetase does. In either lineage, histidyl-tRNA synthetase is likely to have evolved to avoid misrecognizing noncognate tRNAs possessing G_{73}, such as $tRNA^{Asp}$ and $tRNA^{Asn}$, both sharing two other recognition elements, the first and second letters of the anticodon, with $tRNA^{His}$. In this context, the present study shows the evolutionary direction of the molecular recognition concomitant with the evolution of tRNAs.

Judging from the two $tRNA^{His}$ recognition systems, the responsibility of the irregular secondary structure for the histidine specific aminoacylation seems universal. We believe that $tRNA^{His}$ acquired this unique structure in the early stage of evolution, and the inevitable importance for histidylation led to the universal occurrence of the extra nucleotide, notwithstanding the variety of $tRNA^{His}$ processing.

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