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Strategy for RNA Recognition by Yeast Histidyl-tRNA Synthetase

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Abstract—Histidine aminoacylation systems are of interest because of the structural diversity of the RNA substrates recognized by histidyl-tRNA synthetases. Among tRNAs participating in protein synthesis, those specific for histidine all share an additional residue at their 5′-extremities. On the other hand, tRNA-like domains at the 3′-termini of some plant viruses can also be charged by histidyl-tRNA synthetases, although they are not actors in protein synthesis. This is the case for the RNAs from tobacco mosaic virus and its satellite virus but also those of turnip yellow and brome mosaic viruses. All these RNAs have intricate foldings at their 3′-termini differing from that of canonical tRNAs and share a pseudoknotted domain which is the prerequisite for their folding into structures mimicking the overall L-shape of tRNAs.

This paper gives an overview on tRNA identity and rationalizes the apparently contradictory structural and aminoacylation features of histidine-specific tRNAs and tRNA-like structures. The discussion mainly relies on histidylation data obtained with the yeast synthetase, but the conclusions are of a more universal nature. In canonical $tRNA^{His}$, the major histidine identity element is the 'minus' 1 residue, since its removal impairs histidylation and conversely its addition to a non-cognate $tRNA^{Asp}$ confers histidine identity to the transplanted molecule. Optimal expression of histidine identity depends on the chemical nature of the −1 residue and is further increased and/or modulated by the discriminator base N_{73} and by residues in the anticodon. In the viral tRNA-like domains, the major identity determinant −1 is mimicked by a residue from the single-stranded L1 regions of the different pseudoknots. The consequences of this mimicry for the function of minimalist RNAs derived from tRNA-like domains are discussed.

The characteristics of the histidine systems illustrate well the view that the core of the amino acid accepting RNAs is a scaffold that allows proper presentation of identity nucleotides to their amino acid identity counterparts in the synthetase and that different types of scaffoldings are possible. © 1997 Elsevier Science Ltd.

Introduction

Aminoacyl-tRNA synthetases (aaRSs) catalyze the attachment of specific amino acids to transfer RNAs (tRNAs). The overall phenomenology accounting for efficient recognition and catalysis has been partly unraveled and appears to be of similar type in its basic principles (two-step tRNA aminoacylation mechanisms and identity rules) for all canonical systems studied so far.[1,2] This is in line with the common tertiary structure of tRNAs and their involvement in protein synthesis. Nevertheless, certain mechanistic and structural peculiarities differentiate tRNA aminoacylation systems. In particular, aaRSs exhibit differences which lead to their classification into two families,[3,4] and some of them are able to aminoacylate RNA substrates presenting pronounced structural peculiarities. These unusual RNA substrates of synthetases include histidine-specific tRNAs which all possess an additional residue at their 5′-end as compared to other canonical tRNAs,[5] mitochondrial tRNAs which can lack entire structural domains (e.g., refs 6 and 7), and tRNA-like molecules not involved in protein synthesis but in viral replication (reviewed in refs 8 and 9). Thus, HisRS, TyrRS, and ValRS aminoacylate the 3′-end of plant viral RNAs[8–10] and AlaRS attaches an alanine residue to a 10Sa RNA from *Escherichia coli* involved in the degradation of truncated proteins synthesized from damaged messenger RNA lacking a stop codon.[11,12] Interestingly, other tRNA-like domains are recognized by synthetases without being aminoacylated, such as a tRNA-like fold in the promoter region of the messenger RNA of ThrRS, which is recognized by ThrRS.[13]

Here we focus our attention on HisRS from the yeast *Saccharomyces cerevisiae*, a class II synthetase, able to charge two families of RNA substrates all presenting peculiar structural features. This synthetase efficiently aminoacylates not only its cognate $tRNA^{His}$ but also a macro-substrate corresponding to the tRNA-like domain from tobacco mosaic virus (TMV) RNA.[14,15] The ability of HisRS to recognize non-classical substrates has been further extended to the viral tRNA-like structures from turnip yellow mosaic virus (TYMV),[16] brome mosaic virus (BMV),[17] and the satellite virus of TMV (STMV).[15] Figure 1 presents these substrates for HisRS and highlights their peculiar foldings which are

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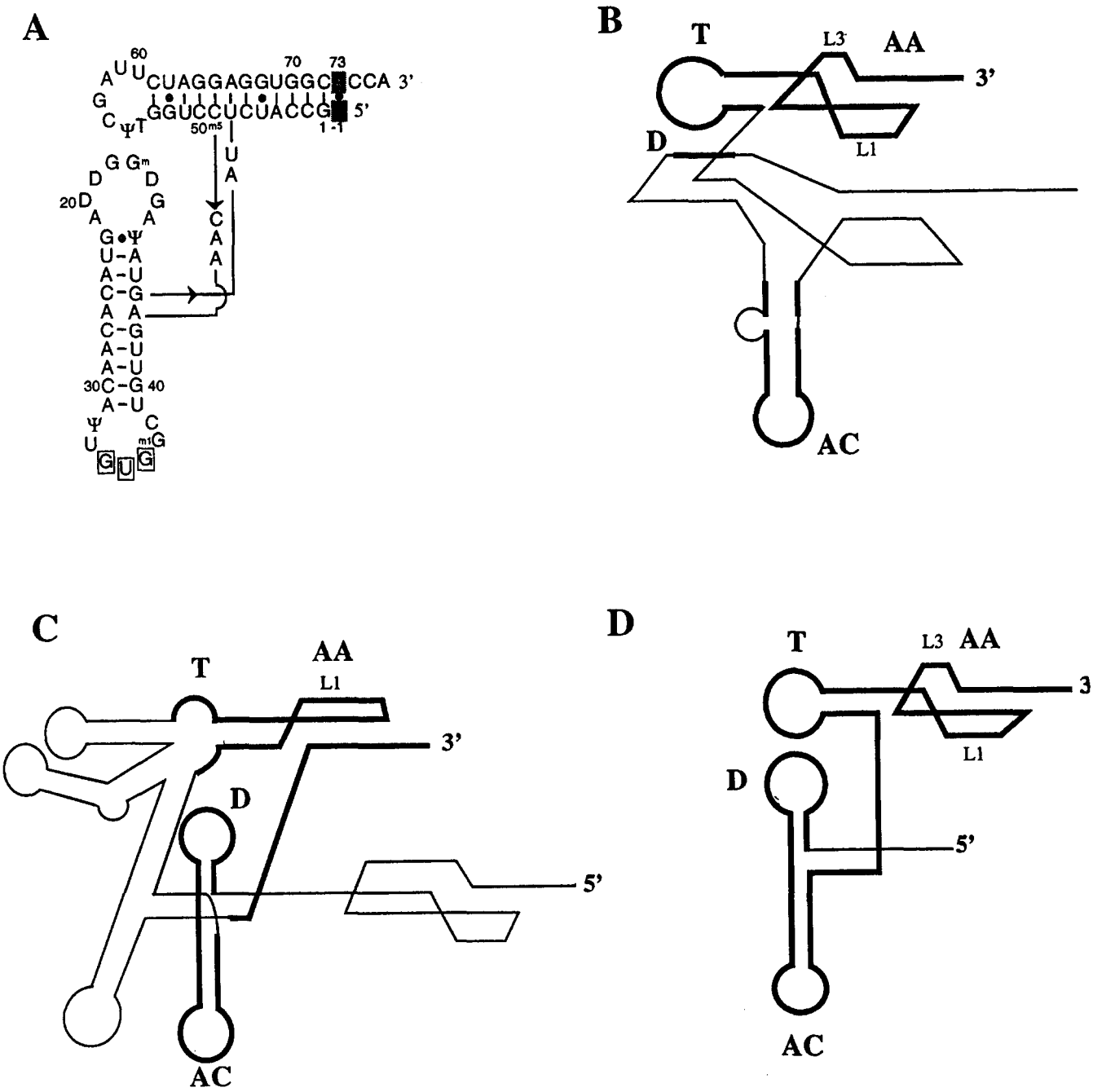

Figure 1. Various RNA substrates for yeast HisRS. The foldings are displayed in such a way to emphasize the L-shape 3-D conformation of tRNAs. (A) Sequence of yeast $tRNA^{His}$ with the anticodon triplet in squares and pair G_{-1}–A_{73} pair shaded. (B) Foldings of the 3′-ends of RNAs from (B) TMV, (C) BMV and (D) TYMV emphasizing the tRNA-like domains (adapted from refs 39–42). Bold lines indicate the analogy with the L-shaped tRNA folding. Thin lines in the tRNA-like structures are extra-sequences not relevant to the aminoacylation properties of the molecules. AA stands for amino acid acceptor arm, T for T-stem and loop, D for D-stem and loop and AC for anticodon arm. L1 and L3 refer to the loops belonging to the pseudoknot which connect the different helices of TMV, BMV and TYMV tRNA-like molecules.[47,48]

displayed in such a way to emphasize an L-shaped 3-D-conformation in each of them. The remarkable gross features characterizing these structures are, for example, different lengths of the sequences forming the 'tRNA-like' structures, different connections between the two branches of the L-shaped structures of the four RNAs, the presence of intervening sequences not participating in the tRNA-like domain and of pseudoknots in the three tRNA-like structures (see below for further details).

To understand how yeast HisRS can accommodate such structurally different RNA substrates, we will describe and compare the histidylation properties of the natural histidine-accepting RNAs and of a series of their variants. This will summarize the present knowledge

on the identity elements required by the eukaryotic yeast HisRS for efficient recognition and charging of its various RNA substrates. Particular emphasis will be given to the functional role of several structural features, especially to the extra-residue at the 5′-end of the canonical $tRNA^{His}$ and to the mimicry of such a residue in the pseudoknots forming the acceptor domain in tRNA-like structures. The universality of the recognition strategies of HisRSs from other phylogenies will be discussed.

Concept of identity for tRNA aminoacylation

Identity elements of tRNAs are defined as an assortment of signals allowing their specific recognition and aminoacylation by their cognate aaRSs. In vivo and in vitro investigations have assigned identity sets for a large number of tRNAs. These sets consist in limited numbers of nucleotides or even specific functional groups acting as positive signals (or determinants) for the cognate synthetase and negative signals (or anti-determinants) preventing recognition by non-cognate synthetases (reviewed in refs 2, 18, and 19). Positive elements are best known and are dispatched in the whole tRNA structure with a preference for two regions, the anticodon loop and the amino acid acceptor arm (Fig. 2A). In some tRNAs, however, the main identity elements are concentrated in the CCA_{OH}-containing acceptor domain. This allows one to engineer small RNA molecules mimicking the amino acid acceptor branch, and consequently enable efficient aminoacylation (reviewed in ref 20).

It became evident that recognition also includes structural signals after transplantation experiments of identity sets into various host tRNAs. Indeed, simple transplantations did not always lead to optimal amino acid acceptance by the host tRNAs; an optimal efficiency was often observed only after structural remodeling of the host molecule (e.g., refs 21–23). Mutational analyses of in vitro transcripts have confirmed the influence of tRNA tertiary structure and stability on aminoacylation by some aaRSs.[24,25]

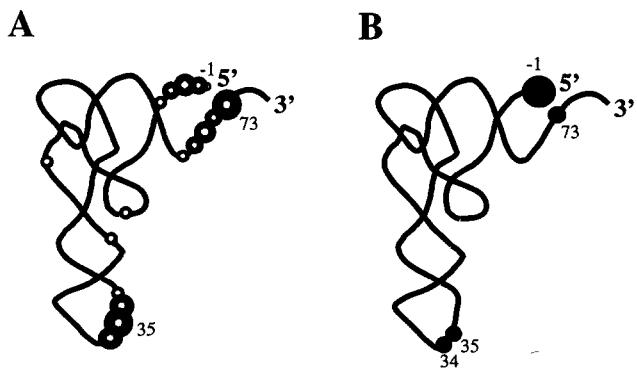

Figure 2. Topology of aminoacylation identity elements in the 3-D structure of tRNAs. Only the backbone is represented. (A) Summary of the known identity positions for the 20 amino acid specificities (indicated by spheres). Frequency of the involvement of nucleotides in identity is proportional to the size of the spheres. (B) Localization of the yeast histidine identity elements by black spheres. Here the size of the spheres is proportional to the strength of the identity.

The determination of the crystal structures of the *E. coli* $tRNA^{Gln}$/GlnRS[26] and yeast $tRNA^{Asp}$/AspRS[27] complexes also highlighted the contribution of RNA flexibility to the formation of cognate complexes. Thus, drastic conformational changes of the tRNAs are observed after complex formation that facilitate direct contacts of the tRNA identity elements with their amino acid counterparts in the proteins. In the aspartate system, the overall conformation of the L-shaped $tRNA^{Asp}$ is more pinched, and the anticodon loop undergoes a large conformational change with a complete unstacking of the bases.[28] In the glutamine system a drastic event occurs at the end of the $tRNA^{Gln}$ acceptor stem in which the last base-pair is disrupted and the CCA_{OH}-end bends backwards to form a hairpin loop.[26]

In a global view, the requirements for specific aminoacylation of tRNAs by aaRSs include not only constraints (e.g., the presence of specific identity elements, structural features) but also relative tolerance (e.g., aminoacylation of minihelices corresponding to tRNA acceptor branches). This is in line with the fact that other flexible or extra-large RNA molecules can adjust to synthetases and become aminoacylated.

A particular strategy for $tRNA^{His}$ recognition

Comparison of the $tRNA^{His}$ species sequenced to date, and covering all phylogenic kingdoms, reveals the presence of an additional nucleotide at the 5′-end of the amino acid acceptor branch. All 12 sequenced $tRNA^{His}$ species, comprising five prokaryotic, six eukaryotic and one mitochondrial species, possess a G_{-1} residue.[5] In prokaryotes, this extra nucleotide is encoded by the gene and comes from an unusual processing by RNase P,[29,30] while in eukaryotes it is added post-transcriptionally.[31] This structural feature makes the additional residue an obvious candidate for specifying histidine identity. This nucleotide, in addition, is opposite to the so-called discriminator base 73,[32] which was shown to participate in defining many tRNA identities.[2,18,19] Thus, residue G_{-1} in combination with nucleotide 73, was proposed to specify histidine identity. Moreover, the nature of these nucleotides distinguishes prokaryotic (G_{-1}–C_{73}) and eukaryotic (G_{-1}–A_{73}) histidylation systems.

The theoretical predictions for a role of nucleotides −1 and 73 in histidine identity were verified by in vitro and in vivo experiments in *E. coli* as well as in yeast. The importance of the extra residue G_{-1} of *E. coli* $tRNA^{His}$ is unambiguous since variants lacking this residue are poor substrates for HisRS. Base replacement at position 73 also causes a marked effect on aminoacylation by HisRS indicating a contribution of this residue to histidine identity. The tuning of this identity is even refined by combining the action of −1 residue with that

of the discriminator base.[33] These conclusions were confirmed by studying minihelices of 13 base-pairs and microhelices of eight base-pairs that reconstruct the acceptor-TC and the acceptor stem of *E. coli* $tRNA^{His}$, respectively. These small RNA molecules can be specifically charged by the prokaryotic enzyme, showing the importance of both N_{-1} and N_{73} elements for histidine identity.[34] Further, introduction of the single base-pair G_{-1}–C_{73} into mini- and microhelix^{Ala} confers histidine acceptance to these molecules.[34] Complementary in vivo experiments pointed out the role of C_{73} as an identity determinant for *E. coli* HisRS.[35]

Concerning the eukaryotic kingdom, nature has retained a similar strategy for discriminating $tRNA^{His}$ among the other tRNAs. We and others showed by in vitro experiments that residue −1 is crucial for histidine identity in yeast since a G_{-1} deficient variant of $tRNA^{His}$ has its catalytic efficiency for histidylation dramatically affected.[36,37] Interestingly, and in agreement with the general idea that transplantation of identity elements from one context to another confers to the host tRNA the new identity, we found that addition of a G_{-1} nucleotide to $tRNA^{Asp}$ renders this tRNA an efficient histidine acceptor (Table 1). The gain in histidylation catalytic efficiency is 230-fold and this extended $tRNA^{Asp}$ molecule is only 70-fold less efficiently histidylated than a $tRNA^{His}$ transcript. These kinetic effects are characterized by changes in K_m and especially in V_{max}, indicating that the G_{-1} residue is involved in both the binding of the tRNA to the synthetase and the catalytic mechanism of histidylation. Residue A_{73}, which forms a mismatch with G_{-1}, plays a less important role in histidine identity.[36]

Anticodon residues in tRNAs are often identity elements (Fig. 2A). Introduction of mutations at anticodon positions in $tRNA^{His}$ affects differentially its histidylation properties. Whereas variants mutated at positions 34 and 35 exhibit losses ranging from 14- to 70-fold as compared to the wild-type yeast $tRNA^{His}$ transcript,[37] mutations at position 36 do not markedly affect the histidylation capacity of the variants.[36,37] Interestingly, mutation of the strategic anticodon positions leads mainly to decreased K_m values, suggesting that anticodon identity positions in yeast $tRNA^{His}$ contribute essentially to better binding of the tRNA to the synthetase. This contrasts with mutations at the amino acceptor stem of $tRNA^{His}$, especially for some variants with Pur_{-1}–Pur_{73} pairs, where the strongly reduced activity is almost exclusively due to reduction in V_{max}.[37]

Recognition of yeast and *E. coli* histidine-specific tRNAs by their cognate synthetases is similar. The G_{-1} residue is the major determinant and the additional contribution of residue 73 is stronger in prokaryotes than in eukaryotes. Further, the base requirement at N_{-1} and N_{73} positions is less constrained in yeast than in *E. coli*. In eukaryotes, anticodon residues are weak histidine identity elements that just increase and modulate the effects of the major determinants. These characteristics are summarized in Figure 2(B), which emphasizes the primordial and specific role of residue G_{-1} and the refinement of the specificity by the participation of both discriminator base 73 and anticodon residues 34 and 35.

This recognition scheme is in agreement with conclusions arising from inspection of the crystallographic structure of free *E. coli* HisRS, namely that the $tRNA^{His}$ acceptor stem may contact the catalytic cleft of the protein and that the anticodon stem may be packed against the C-terminal domain of HisRS.[38]

The unexpected histidine identity of tRNA-like domains from viral RNAs

Some plant viruses possess genomic RNAs which can be aminoacylated at their 3′-ends by host or heterologous aminoacyl-tRNA synthetases (reviewed in refs 8 and 9). Only a limited number of aaRSs have been found to charge these RNAs. ValRS recognizes tymoviral RNAs, TyrRS charges bromo-, cucumo-, and hordeiviral RNAs, and HisRS aminoacylates tobamoviral RNAs (with one exception). Due to their functional similarities with tRNAs (except for their non-involvement in protein synthesis as amino acid donors), the 3′-end of these molecules, referred to as 'tRNA-like', are proposed to structurally resemble canonical tRNAs. Based on chemical and enzymatic probing experiments, combined with graphical modeling and knowledge of crystallographic tRNA structures, 3-D models of at least one member of each tRNA-like family have been proposed.[39–41] As expected, RNA domains mimicking L-shaped tRNA structures could be visualized in the three models studied so far. Each model is based on a novel folding topology different from that accounting for the 3-D structure of canonical tRNAs. These alternate

Table 1. Influence of residue G_{-1} on the histidylation by yeast HisRS of transcripts derived from wild-type yeast $tRNA^{His}$ and $tRNA^{Asp}$

tRNAs	K_m (μM)	V_{max} (arbitrary units)	V_{max}/K_m (arbitrary units)	L	G
$tRNA^{His}$	0.8	2650	3310	1	–
$tRNA^{His}$ **(− G_{-1})**	3.6	25	7	470	–
$tRNA^{Asp}$	1.5	0.3	0.2	16,600	1
$tRNA^{Asp}$ **(+ G_{-1})**	0.9	42	4.7	70	230

Aminoacylation conditions are as in ref 36. L and G correspond to losses and gains of specificity, respectively. L is expressed by $(V_{max}/K_m)_{tRNA^{His}}/(V_{max}/K_m)_{variant}$ and G by $(V_{max}/K_m)_{variant}/(V_{max}/K_m)_{tRNA^{Asp}}$. Experimental errors on V_{max} and K_m values varied at most by 15%.

topologies require the mandatory presence of a pseudoknot in the amino acid acceptor branch of the RNA (see Fig. 1) and also include other structural features absent in canonical tRNAs (e.g., ref 42).

Considering the rules specifying histidine identity to $tRNA^{His}$ and the structural characteristics of tRNA-like structures, it appears surprising a priori that they share the potential to be charged specifically by yeast HisRS, since they do not show any apparent -1 identity residue, nor canonical anticodon loop or histidine anticodon (except in some TMV strains and STMV RNAs), that might be substitutes for the canonical identity elements. Despite these differences with $tRNA^{His}$, the tRNA-like structures from tobamoviruses, such as the TMV RNA and the STMV RNA,[14,15] are histidylated with catalytic specificities only slightly reduced as compared to those of $tRNA^{His}$ (Table 2). Those of TYMV and BMV primarily known to be tyrosine and valine acceptors, respectively, are 'mischarged' by yeast HisRS,[16,43] but with a histidylation potential less pronounced than that of TMV and STMV RNAs (Table 2). Histidylation of the latter molecules is sensitive to experimental conditions (more specifically to the $[Mg^{2+}]/[ATP]$ ratio) and charging levels at the plateau reach approximately 25% for TYMV RNA and 40% for BMV RNA. In the case of TYMV, the histidylation properties of RNA molecules of various lengths have been tested. The shortest RNA fragment able to fold into an L-shaped structure (88 nucleotides) is the best substrate for the enzyme, indicating that this fragment contains all the requirements for efficient recognition by yeast HisRS.[16] The longest TYMV tRNA-like structures display less efficient catalytic constants, suggesting that the 5′-flanking region has a negative influence on the histidylation reaction.

An easy way to explain the common aminoacylation properties for canonical tRNAs and tRNA-like structures would be to assume that histidine identity is governed by different rules in the two types of molecules. However, because of the chemical rationale underlying biological processes, this view is unlikely and it is believed that the histidylable tRNA-like structures should contain a structural mimic of the major N_{-1} histidine determinant in canonical tRNAs.

Molecular mimicry of the histidine identity rules in tRNA-like domains

Differences between canonical $tRNA^{His}$ species and histidine-accepting tRNA-like molecules concern their sequences as well as their structures, although an analogue of an L-shape domain has been identified in all tRNA-like structures studied so far. Except for the common CCA_{OH} sequence present at the 3′-end of tRNA and tRNA-like structures, no consensus sequences are present elsewhere in the molecules. Furthermore, the discriminator base or its equivalent is not conserved (either an A or a C) (Table 3); the sequence of the anticodon of $tRNA^{His}$ or of the pseudoanticodon found in tRNA-like structures also varies among all the reported histidylable molecules (Table 3). Another striking difference concerns the number of base-pairs forming the amino acid acceptor branch. All histidine-specific tRNAs possess an extended branch (13 base-pairs) due to the extra pair between the additional residue and discriminator base 73, as is also the case for TYMV and BMV tRNA-like structures. TMV and STMV tRNA-like structures present only 12 base-pairs in their acceptor branch (11 base-pairs plus the analogue of the N_{-1}–N_{73} pair) (Table 3). In addition, all chargeable tRNA-like structures share peculiar foldings, with the pseudoknots forming a quasi-continuous double-helix in their amino acid acceptor branches (see Figs 1B–D and 3). This structural element is of tertiary nature and likely involves base-pairing between nucleotides of a loop region with residues outside that loop. Thus, a pseudoknot has two double-helical stem regions S1 and S2 connected by two loops called L1 and L3.[46–48] The tRNA-like molecules discussed here have either 2, 3, or 4 nucleotides in loop L1, and 2, 3, or 94 nucleotides in loop L3 (Table 3).[49] It appears that these variations in the lengths of the two connecting loops do not perturb histidylation and thus do not act as negative elements against yeast HisRS. However, mutational analysis of their sequence combined with aminoacylation

Table 2. Histidylation by yeast HisRS of its canonical $tRNA^{His}$ and of tRNA-like structures of plant viruses

RNA substrate origin	Size (number nt)	$[Mg^{2+}]/[ATP]$[a]	pH	Temperature (°C)	Charging plateau[b] (%)	L (*x*-fold)
Yeast $tRNA^{His}$[c]	77	–	–	–	100	1
TMV genomic RNA	6395	2	7.8	30	98	4.7
STMV genomic RNA	1059	2	7.8	30	30	17
BMV tRNA-like domain	201	15	8.1	37	40	438
TYMV enlarged tRNA-like domain	264	15	8.5	30	25	100

Aminoacylation of tRNA-like structures was done either on the entire viral RNA or on the isolated tRNA-like domain. Charging levels were calculated assuming all RNA molecules are active for aminoacylation. Charging conditions of the different tRNA-like structures are as described for TMV,[41] STMV,[15] BMV,[43] TYMV[16] RNAs. L-values are losses of specificity and are expressed as outlined in Table 1.

[a]For the ratio of 15, the magnesium concentration was kept constant and ATP concentration varied.

[b]Charging levels less than 100% reflect the equilibrium between the aminoacylation and the deacylation reactions.[44,45]

[c]Aminoacylation of yeast $tRNA^{His}$ was performed in all the mentioned experimental conditions to permit the comparison with the different tRNA-like structures. Experimental errors on V_{max} and K_m values varied at most by 15%.

Table 3. Summary of structural characteristics of RNAs charged by yeast HisRS

RNAs	Length of amino acid accepting branch (Number bp)	Pseudoknot organization L1 L3 (Number nt)	Sequence of pseudo-anticodon	Major His identity nucleotides N_{-1} N_{73} or their mimics
Yeast tRNAHis	13	— —	–GUG–	G_{-1} A_{73}
TMV/mini-TMV	12	3 2	–GUU–[a]	A_{18} C_4
STMV	12	3 3	–GUG–	A_{19} C_4
BMV/mini-BMV	13	2 94/0[b]	–ACA–	A_{117} A_4
TYMV/mini-TYMV	13	4 3	–CAC–	$U_{21/22}$[c] A_4

[a]Anticodon is GUG in some TMV strains.[8]
[b]The L3 loop is extended in the entire tRNA-like structure and is absent in mini-BMV RNA (see refs 49 and 54 and Figs 1 and 4, for details).
[c]For transcriptional reasons, the pseudoknot of mini-TYMV has been modified from that of TYMV RNA: the acceptor helix is built up of three successive helices of 3, 4, and 5 base-pairs instead of 4, 3, and 5. As a consequence, the residue located in front of A_4 is numbered 21 for the TYMV RNA and 22 for the TYMV minihelix (see ref 16).

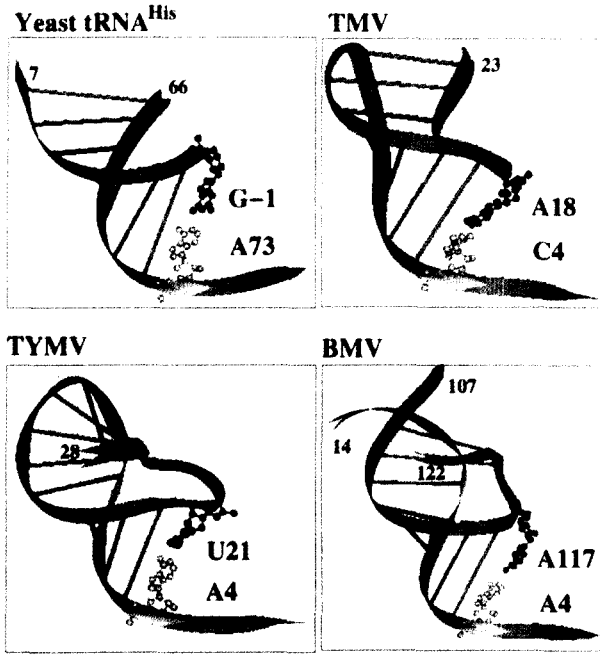

Figure 3. Structural mimicry between the histidine identity elements in canonical tRNA and in viral tRNA-like structures. The four panels display, in a similar orientation, models of the terminal parts of the histidine-accepting branches of the RNAs (seven to nine base-pairs and the NCCAOH 3′-stretch). The graphic representations were produced with the DRAWNA software.[50] The G_{-1} major determinant in $tRNA^{His}$ and its A_{18}, U_{21}, and A_{117} mimics in the three tRNA-like molecules are colored in red; the discriminator residue A_{73} in tRNAHis and its C_4 and A_4 mimics are colored in yellow. The RNA backbones are in green. Numbering of residues is as in $tRNA^5$ or in the entire tRNA-like molecules.[39–41,51] For STMV RNA, the model would be similar as for TMV RNA. The figure emphasizes the similar positioning of the N_{-1}–N_{73} pairs in the four RNAs.

assays will be required to assess their direct contribution in histidylation efficiency.

As anticipated above, similarities between canonical $tRNA^{His}$ and tRNA-like structures concerning their identity elements have been identified. The topology of pseudoknots forming the amino acceptor branches of the tRNA-like structures emerging from molecular modeling[39–41] is such that a nucleotide belonging to loop L1 is stacked on top of the major helix and thus can mimic the location and function of residue −1 in canonical $tRNA^{His}$ (Fig. 3). As is the case for G_{-1} in $tRNA^{His}$, this nucleotide faces the equivalent of residue 73 in tRNA-like structures. This property within the pseudoknot was already described in the case of the valylatable TYMV RNA before knowing the rules of histidine identity.[39] Thus, mimics of nucleotides G_{-1} and N_{73} are identified in TMV, STMV and BMV tRNA-like structures and are good candidates to account for the histidylation properties of these molecules. Interestingly, in all histidylable tRNA-like structures studied so far, the equivalent of the nucleotide −1 is not a G residue as found in all histidine canonical tRNAs, but either an A residue for the TMV, STMV, and BMV tRNA-like domains (numbered A_{18}, A_{19}, and A_{117}, respectively),[51] or a U residue for the TYMV tRNA-like (U_{21}) (Table 3).

Engineering of histidine-accepting RNA structures

The proof of the role of residue −1 as a mimic of the major histidine identity elements came from two complementary series of experiments. First, it was shown that minisubstrates derived from various tRNA-like domains and mimicking an amino acceptor branch are still substrates for HisRS, and second that mutations of the equivalent −1 nucleotide in these minihelices affect the histidylation efficiency.

Minihelices recapitulating the amino acid acceptor branch of histidylable tRNA-like structures were designed. Thus, short pseudoknotted fragments of 38, 34, and 42 nucleotides derived from TMV, BMV, and TYMV tRNA-like structures, respectively, were synthesized by in vitro transcription (Fig. 4) and their ability to be charged by yeast HisRS tested. As expected, all these minisubstrates can be histidylated. The highest plateau level was found with a minihelix derived from TMV RNA where 40% of the RNA molecules can be histidylated. In contrast, a minimalist RNA structure derived from BMV RNA is only aminoacylated up to

Table 4. Histidylation of minimalist tRNA-like domains

RNAs	Length (nt)	$[Mg^{2+}]/[ATP]^a$	pH	Temperature (°C)	Charging plateau[b] (%)	$L^{(1)}$	$L^{(2)}$
Mini-TMV	38	2	7.8	30	40	107	nd
Mini-BMV	34	2	8.1	15	4	34	31,000
Mini-TYMV	42	15	8.5	30	35	0.5	55
Mini-TYMV	42	1.5	7.5	30	4	nd	80,000

Experimental conditions for histidylation are as decribed in refs 15, 16, 36, and 54.
[a,b]See remarks in Table 2.
The temperature for charging the minimalist BMV RNA is lower than that for the other minisubstrates in order to increase the stability of the BMV minihelix which is formed by a bimolecular association of two RNA stretches.[54] $L^{(1)}$ refers to the aminoacylation efficiency of the studied minisubstrate as compared to that of the corresponding entire tRNA-like domain $[(V_{max}/K_m)_{tRNA\text{-}like}/(V_{max}/K_m)_{Mini\text{-}RNA}]$. $L^{(2)}$ corresponds to the comparative aminoacylation efficiency of the studied minisubstrate with that of yeast $tRNA^{His}$ in vitro transcript $[(V_{max}/K_m)_{tRNA^{His}}/(V_{max}/K_m)_{Mini\text{-}RNA}]$. Experimental errors on V_{max} and K_m values varied at most by 15%.

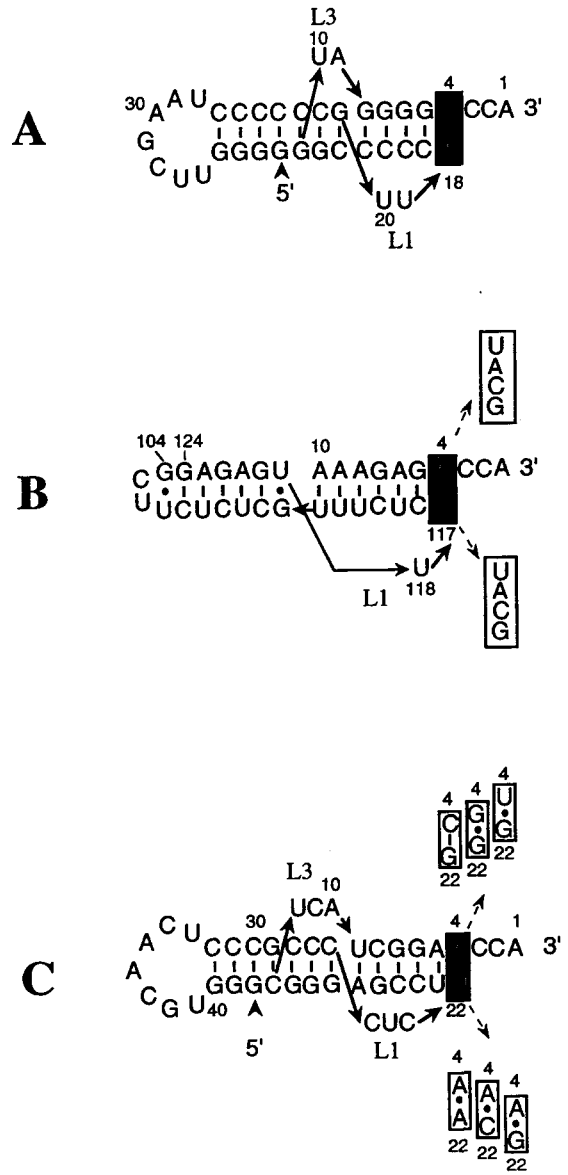

Figure 4. Minimalist substrates of yeast HisRS mimicking the amino acid acceptor branch of TMV RNA (A), BMV RNA (B) and TYMV RNA (C) with the equivalent of pair N_{-1}–N_{73} shaded. Fifteen variants of BMV minihelix and six variants of TYMV minihelix with mutations at pair N_{-1}–N_{73} were synthesized. Numbering of residues is as in the entire tRNA-like structure. Notice the particular topology of the BMV-derived minimalist RNA substrate of HisRS made of two RNAs and thus missing loop L3.[49,54]

4% (Table 4). For the TYMV-derived minihelix, charging was studied as a function of experimental conditions. It was found that histidylation is sensitive to the $[Mg^{2+}]/[ATP]$ ratio, the pH, and the temperature. This leads to plateau levels varying from 4 to 35%.

Minihelices show weak losses in aminoacylation efficiency (from 0.5 to 107) as compared to the entire corresponding tRNA-like domains. This confirms that the major histidine identity elements are located in the amino acid acceptor branch of tRNA-like molecules. When minisubstrates are compared to yeast $tRNA^{His}$, the losses vary in a much larger range. For the minisubstrates derived from BMV RNA, this loss is 31,000-fold, indicating that with the utilized experimental conditions, this molecule is not optimally adapted for synthetase interaction. For histidylation of the TYMV-derived minihelices, losses vary between 55- and 80,000-fold depending on the experimental conditions. A similar effect was recently described for the methionylation of a TYMV tRNA-like variant where the valine identity elements in the anticodon-like loop were exchanged by methionine elements,[52] and is reminiscent to aminoacylation properties of tRNA mischarging reactions.[53] Indeed, mischarging is facilitated at low ionic strength or other conditions that favor a greater plasticity of the tRNA and hence a better adaptation on the synthetase.

A further confirmation of the role of the N_{-1}–N_{73} equivalent pairs came from experiments where homologous residues (A_{22}–U_4 for TYMV-minihelix and A_{117}–A_4 for the BMV-minihelix) were mutated into several combinations, as highlighted in Figure 4 (refs 36 and 51). Histidylation properties were affected in both types of molecules. In the case of the TYMV minihelices, the variant (G_{22}–A_4) containing the identity combination from eukaryotic $tRNA^{His}$ is the most efficient substrate for the yeast enzyme and shows a 10-fold increased efficiency as compared to the wild-type minihelix.[36] Details concerning minisubstrates derived from the BMV tRNA-like structure will be published elsewhere.[54]

Conclusions

This paper rationalizes the present knowledge about the recognition of RNAs by HisRS, and particularly by the yeast synthetase, and highlights how this enzyme can accommodate not only its canonical $tRNA^{His}$ but also tRNA-like domains presenting intricate architectures. These data illustrate the view that tRNA-like structures are scaffolds selected by nature to present histidine identity elements to HisRS, in a way mimicking the situation in the cognate $tRNA^{His}$. The consequence of this mimicry is the similarity of the rules governing histidine identity in both structural contexts. This conclusion is in line with former results on valine identity of the TYMV tRNA-like structure. Indeed, equivalent nucleotides to the anticodon residues of $tRNA^{Val}$ in the TYMV tRNA-like structure are valine identity elements.[55,56] This view on identity rules appears to be more general and explains well the functionality of the tRNA-like molecules studied so far.[12,13,52]

Concerning histidine identity more precisely, our studies demonstrate that the presence of and precise location of the N_{-1}–N_{73} identity pair, particularly of the −1 residue is more important than the chemical nature of these residues. Indeed, HisRS is able to charge molecules presenting different combinations at these positions (G–A in $tRNA^{His}$, A–C in TMV-, STMV-, BMV-RNA, and U–A in TYMV-RNA). The chemical nature of the determinants, however, plays a functional role in tuning the strength of the histidine identity. Because the histidine identity set is rather simple, it is likely that other RNA molecules found in nature, but not yet discovered, present structural features enabling them to interact with HisRS. Good candidates are RNA molecules containing pseudoknotted domains. Non-natural molecules with such properties might further be obtained by in vitro selection procedures. Such molecules, or their variants, could become interesting blocking agents of HisRSs, for example as 'RNA antibiotics' in inhibition strategies of aminoacylation systems.

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