IUBMB *Life*, 49: 341–351, 2000
Copyright © 2000 IUBMB
1521-6543/00 $12.00 + .00

Critical Review

Pseudouridine in RNA: What, Where, How, and Why

Michael Charette and Michael W. Gray
Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Summary

Pseudouridine (5-ribosyluracil) is a ubiquitous yet enigmatic constituent of structural RNAs (transfer, ribosomal, small nuclear, and small nucleolar). Although pseudouridine (Ψ) was the first modified nucleoside to be discovered in RNA, and is the most abundant, its biosynthesis and biological roles have remained poorly understood since its identification as a "fifth nucleoside" in RNA. Recently, a combination of biochemical, biophysical, and genetic approaches has helped to illuminate the structural consequences of Ψ in polyribonucleotides, the biochemical mechanism of U→ Ψ isomerization in RNA, and the role of modification enzymes (Ψ synthases) and box H/ACA snoRNAs, a class of eukaryotic small nucleolar RNAs, in the site-specific biosynthesis of Ψ. Through its unique ability to coordinate a structural water molecule via its free N1-H, Ψ exerts a subtle but significant "rigidifying" influence on the nearby sugar-phosphate backbone and also enhances base stacking. These effects may underlie the biological role of most (but perhaps not all) of the Ψ residues in RNA. Certain genetic mutants lacking specific Ψ residues in tRNA or rRNA exhibit difficulties in translation, display slow growth rates, and fail to compete effectively with wild-type strains in mixed culture. In particular, normal growth is severely compromised in an *Escherichia coli* mutant deficient in a pseudouridine synthase responsible for the formation of three closely spaced Ψ residues in the mRNA decoding region of the 23S rRNA. Such studies demonstrate that pseudouridylation of RNA confers an important selective advantage in a natural biological context.

IUBMB ***Life*, 49: 341–351, 2000**

Keywords Base stacking; box H/ACA snoRNA; hydrogen bonding; pseudouridine; pseudouridine synthase; RNA; water bridge.

INTRODUCTION

Of the many intriguing features of RNA, none is more puzzling than the large number of structurally diverse residues (modified nucleosides) it contains (*1*). The first such modified nucleoside to be discovered, and the most abundant in RNA, is pseudouridine (Ψ), the 5-ribosyl isomer of uridine (U) (*1*; see Fig. 1). A recent influx of information about Ψ is providing structural insights and new experimental tools as well as data on the intriguing pattern of occurrence of Ψ in various RNA species (tRNA,[1] rRNA, snRNA, and snoRNA), the unusual mode of selection of U residues for conversion to Ψ, and, most importantly, possible biological roles of Ψ in RNA (for detailed perspectives on the history, chemistry, and biology of Ψ, see *1–8*).

STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF PSEUDOURIDINE

Early speculation about possible roles for Ψ in RNA (see *1*, *2*) was centered on its distinctive physicochemical properties in comparison with its parent nucleoside, U. Ψ is unique among modified nucleosides in possessing a C-C rather than the usual N-C glycosyl bond that links base and sugar (Fig. 1). Because of the enhanced rotational freedom in C-C compared with N-C glycosyl bonds, Ψ was anticipated to exhibit greater conformational flexibility than U. The fact that the free N1-H in Ψ can act as an additional hydrogen bond donor (Fig. 1) also indicated that Ψ residues might participate in novel pairing interactions in RNA. Finally, it was suggested that the N1-H of Ψ might exhibit a high group transfer potential for acyl moieties (*2*, *9*).

Conformational studies of Ψ as the free nucleoside indicate a slight preference for the *syn* glycosyl conformation vs. the *anti* configuration that U and other nucleosides adopt (*5*, *10*, *11*). Consequently, it has been proposed that Ψ might function as a conformational switch in RNA, given the low energy requirement for the *syn*/*anti* transition and the equivalence, in terms of hydrogen-bonding potential, of N1-H in the *syn* conformer of Ψ and N3-H in the *anti* conformer of U (see *2*, *10*). However, within the context of a polynucleotide chain, Ψ has so far

Received and accepted 15 March 2000.
Address correspondence to M. W. Gray. Fax: 902-494-1355; E-mail: M.W.Gray@Dal.Ca

[1] Abbreviations: ASL, anticodon stem-loop; LSU, large subunit; PTC, peptidyltransferase center; rRNA, ribosomal RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; snoRNP, small nucleolar ribonucleoprotein; SSU, small subunit; tRNA, transfer RNA.

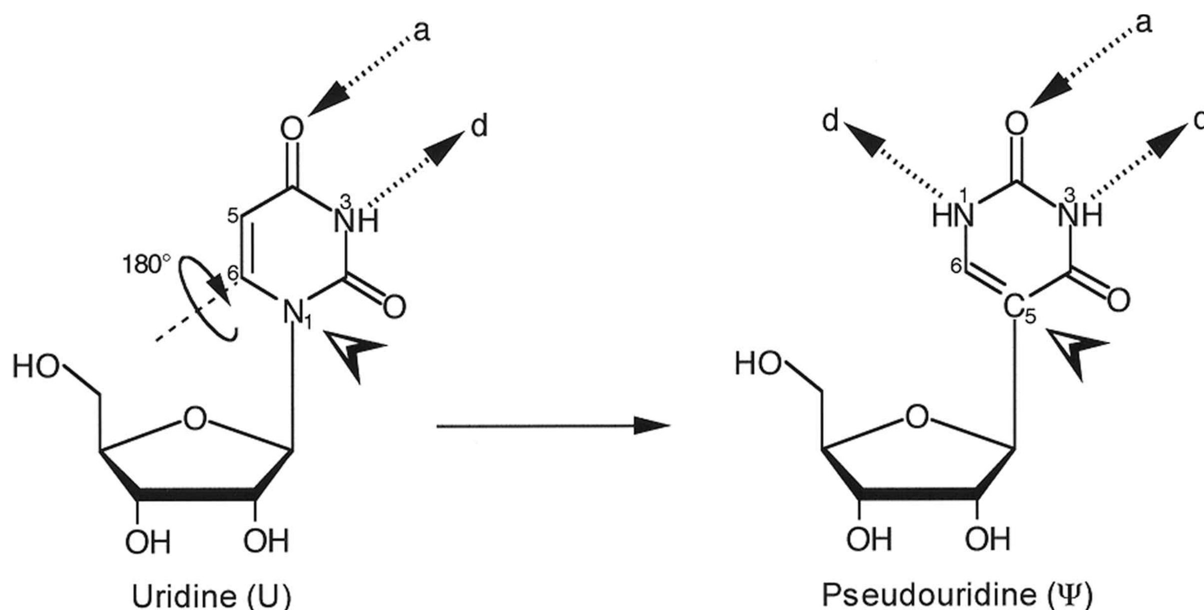

Figure 1. Chemical differences between uridine (U, 1-*β*-D-ribofuranosyluracil) and pseudouridine (Ψ, 5-*β*-D-ribofuranosyluracil), both depicted in the *anti* glycosyl configuration. The uracil base in uridine (left) is linked through its N-1 position (arrowhead) to the C-1′ position of the ribose. The base in U possesses one hydrogen-bond acceptor and one donor (dashed arrows; a and d, respectively). Isomerization occurs when the uracil base is rotated 180° through an N3–C6 diagonal axis (circular arrow). In pseudouridine (right), the C-5 position of uracil (arrowhead) is linked to the C-1′ position of the sugar, resulting in an increase in hydrogen bonding capacity (to one acceptor and two donors) compared with that in U.

been found only in the *anti* configuration (*11*, *12*); moreover, in these cases, Ψ confers rigidity rather than flexibility on both single- and double-stranded regions, even though U and Ψ share the same basic topology in RNA (*5*, *6*, *11*, *12*). This conclusion is based on findings from nuclear magnetic resonance, X-ray crystallography, and molecular dynamics simulations, powerful approaches for determining or inferring the effect of Ψ on RNA structure (see *5*, *6*, *11–16*). The special properties of Ψ relative to U are largely attributable to the former's additional hydrogen-bonding capabilities. In the *anti* conformation, Ψ in RNA provides the appropriate geometry and distance for coordination of a water molecule between its N1-H and the 5′ phosphates of both Ψ and the preceding residue (*6*, *13*). In cases where such a water bridge has been inferred, this restricts base conformation and mobility of the backbone 5′ to the site of pseudouridylation, regardless of sequence or structure (single- or double-strand) context (*11*, *15*) (see Fig. 2).

Pseudouridylation has the additional effect of enhancing local RNA stacking in both single-stranded and duplex regions (*11*, *15*) by favoring a 3′-endo conformation of the ribose, which restricts the base moiety to an axial *anti* conformation (*5*, *11*; see Fig. 2). Here again, a structured water molecule is implicated in the mechanism of this stabilization, which involves replacement of a weak C5-H . . . O_W interaction in U by a stronger N1-H . . . O_W hydrogen bond in Ψ (*5*, *6*). The resulting reduced conformational flexibility of the Ψ moiety renders the phosphodiester backbone in its vicinity more rigid. Consequently, stacking of neighboring nucleosides is cooperatively increased, an effect that is propagated throughout adjacent helical regions. In fact, improved base stacking has been proposed to be the most important contribution of Ψ to the stabilization of RNA structure (*5*, *11*, *12*, *14*, *15*).

DISTRIBUTION AND LOCALIZATION OF PSEUDOURIDINE IN DIFFERENT CLASSES OF RNA

Transfer RNAs

Ψ is found in almost all tRNAs, notably as the nearly universal Ψ55, after which the TΨC stem-loop is named (Fig. 3). Other locations at which Ψ occurs in all three domains of life (archaebacteria, eubacteria, and eukaryotes) as well as in organelles (mitochondria and chloroplasts) include the D stem and the anticodon stem and loop. Ψ is found less frequently at many other sites in tRNA (Fig. 3), in a distribution that is often domain-specific (*6*). It contributes to the stabilization of the specific structural motifs in which it occurs: for example, the TΨC loop (Ψ55), the D stem (Ψ13), the anticodon stem (which often features a strong closing base pair between Ψ39 and A31 [*16*]), and the anticodon loop (noncanonical base-pairing between Ψ38 and residue 32).

Small Nuclear and Nucleolar RNAs

In eukaryotes, Ψ is found in the major spliceosomal snRNAs (U1, U2, U4, U5, and U6) (*7*) and in the minor vertebrate

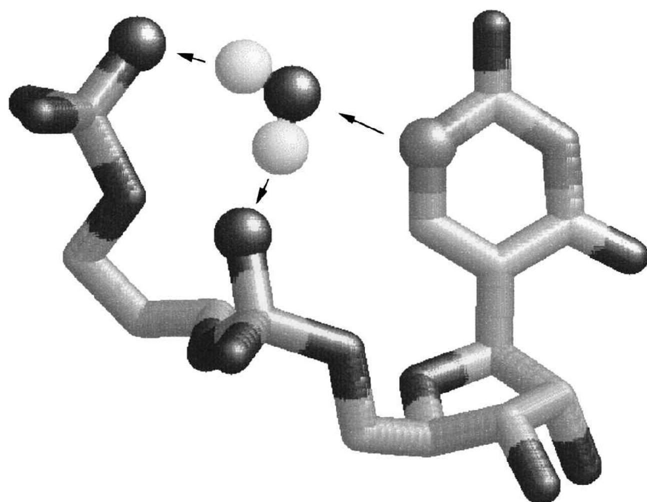

Figure 2. Representation of the hydrogen bond–mediated water bridge involving Ψ in RNA. In this three-dimensional model, a structured water molecule is depicted as forming hydrogen bonds (arrows) with N-1 of Ψ (to the right) and with the sugar-phosphate backbone of both the Ψ and the preceding residue (to the left; only the backbone of the latter residue is shown). The figure was prepared by superimposing a theoretical water molecule (available at http://www2.chemie.unierlangen.de/software/corina/free_struct.html) (*79*) onto the three-dimensional structure of the Ψ39-containing portion of a yeast $tRNA^{Phe}$ (coordinates 1TRA). Modeling was carried out with RasMol-2.6-ucb-beta (*80*), which is available at http://www.umass.edu/microbio/rasmol/.

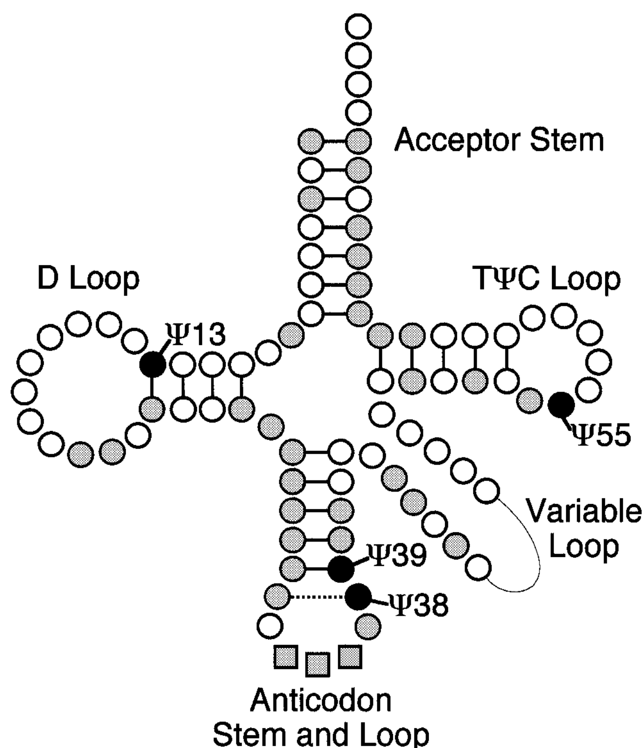

Figure 3. Distribution of Ψ residues in tRNA. Ψ residues found at the same position in tRNAs spanning all three domains of life (eubacteria, archaebacteria, and eukaryotes, including the eubacteria-like organelles, mitochondria, and chloroplasts) are shown as filled circles. Shaded symbols (circles and squares) indicate nucleotide positions at which Ψ residues occur in a more restricted, sometimes domain-specific, fashion. Shaded squares denote the anticodon sequence. The dashed line at the base of the anticodon stem represents a noncanonical ("pseudo") base pair. Nucleotide positions are numbered according to the standard convention for tRNA (as in *6*). Modified from Auffinger and Westhof (*6*).

variants responsible for AU/AC intron splicing (U12, U4atac, and U6atac) (*17*). These Ψ residues are often phylogenetically conserved but with organism- or taxa-specific variations; nearly all are located in functionally important regions that participate in the intermolecular RNA–RNA or RNA–protein interactions involved in the assembly and functioning of the spliceosome (*7*). For example, a Ψ–A pair between U2 snRNA and pre-mRNA is positioned immediately adjacent to the branch site in the intron and may help to stabilize the resulting snRNA/intron helix, thereby facilitating the "bulging out" of the adenosine nucleophile that initiates the first step of the splicing reaction. Ψ residues are often localized in the region of interaction between U4 and U6 snRNA in the presplicing complex and between U1 snRNA and the 5′ splice site.

Ψ residues are also found in snoRNAs, notably U3 (*7*, *18*, *19*), U8, snR4, and snR8 (*20*); as yet, neither the formation nor the function of Ψ in snoRNAs has been investigated in any detail.

Ribosomal RNAs

A ubiquitous constituent of the small subunit (SSU) and large subunit (LSU) rRNAs (*3*, *8*) of eubacteria, archaebacteria, and eukaryotes (including mitochondria and chloroplasts) (*21*), Ψ is also found in 5.8S and a few 5S (*22*) rRNAs. Nucleotide-resolution mapping of Ψ in LSU rRNA, first in *Escherichia coli* (*23*) and subsequently in *Saccharomyces cerevisiae* (*24*), confirmed prior indications (*9*) that Ψ residues cluster in functionally important domains of the LSU rRNA, specifically domains II (near the 5′-end of LSU rRNA), IV (centrally located) and V (near the 3′-end of the molecule) (Fig. 4). Domain V constitutes the peptidyltransferase center (PTC) whereas domain IV is the decoding center of the LSU rRNA, the site of interaction of LSU rRNA with both mRNA and the anticodon stem-loop (ASL) of tRNA. Domains II and IV, although distant from domain V in primary and secondary structure, are in close three-dimensional proximity to the site of peptide bond formation (*23*, *25*). Indeed, Ψ residues such as *E. coli* Ψ2580 (the only site of pseudouridylation in the mitochondrial LSU rRNA of *S. cerevisiae* and probably also human and mouse [*21*]; see Fig. 4) as well as other Ψ residues in the PTC and in domain IV have been mapped to the A- and P-sites of the ribosome (*25*), close to the point of

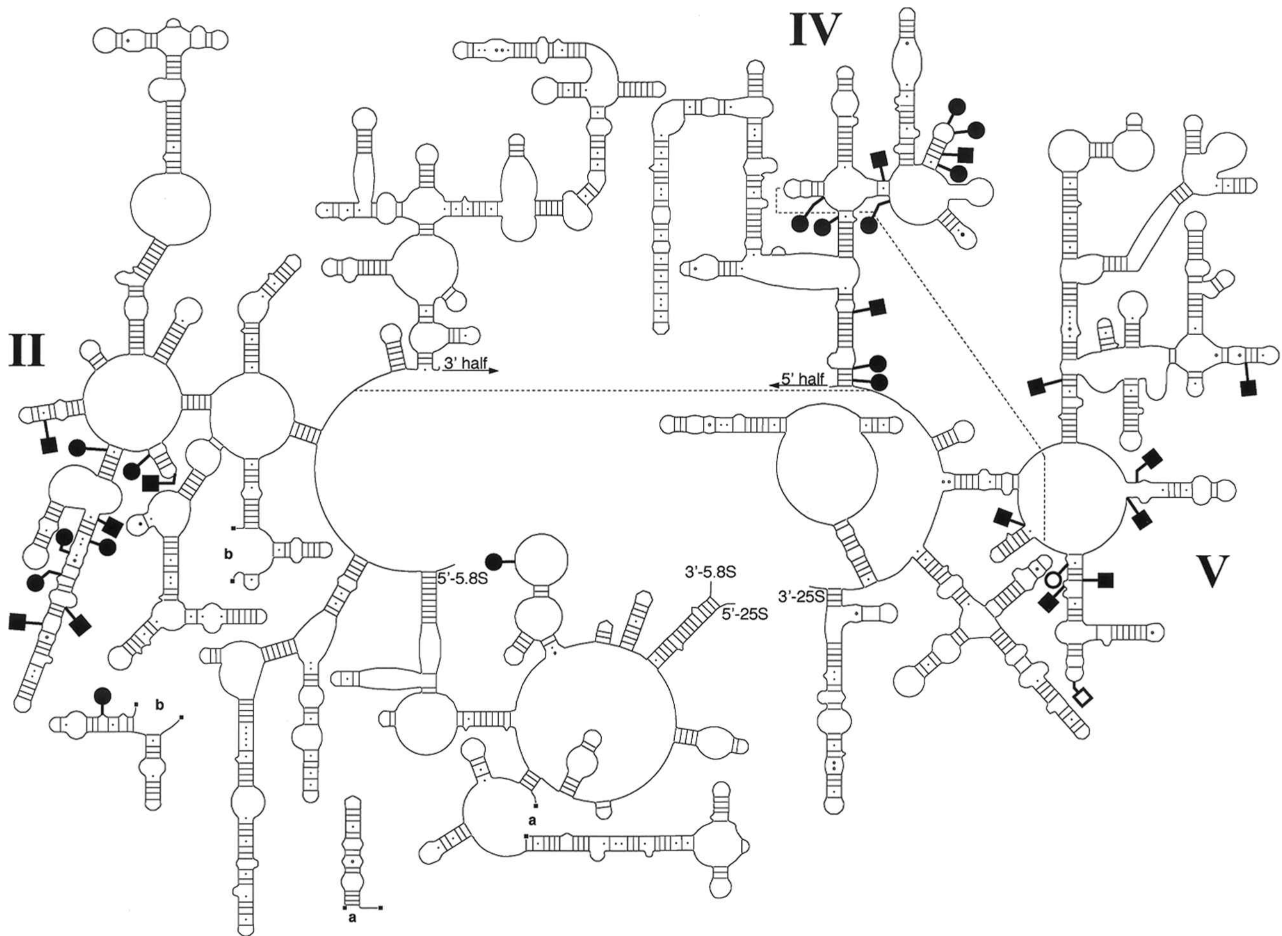

Figure 4. Clustering of Ψ residues (indicated by open and filled circles and squares) in domains II, IV, and V of the cytoplasmic LSU rRNA (5.8S + 25S) of yeast, *Saccharomyces cerevisiae* (*24*, *81*). The open circle denotes the only Ψ residue present in yeast mitochondrial LSU rRNA (this particular residue is not present in yeast cytoplasmic LSU rRNA). Squares indicate the 16 Ψ residues (of the total of 30 in yeast cytoplasmic 25S rRNA) that have been experimentally deleted by disruption of the corresponding box H/ACA snoRNA gene (*8*). The empty square represents a Ψ that had been highlighted (*2*, *9*) as a possible catalytic residue in the peptidyltransferase reaction (see text). Dotted lines represent tertiary interactions that place domains II and IV in the three-dimensional vicinity of the PTC in domain V. The yeast LSU rRNA structure is from Schnare et al. (*82*).

interaction between the PTC and the 3′-end of ribosome-bound aminoacyl- (A site) and peptidyl- (P-site) tRNAs (*26*, *27*). In contrast, no clustering of Ψ residues is observed in known functional regions of SSU rRNA (*3*, *8*).

Although the proportion of Ψ residues in eukaryotic LSU rRNAs (0.9–1.4%) is substantially greater than in their eubacterial, archaebacterial, or organellar counterparts (0.03–0.4%), these additional Ψ residues are still clustered in domains II, IV, and V (*21*). Notably, the low Ψ content in archaebacterial LSU rRNA (*21*, *28*) is more like that seen in eubacteria than in eukaryotes.

In closely related organisms, homologous rRNAs share a clade-specific set of Ψ residues, with organism-specific variations. However, no Ψ residues are universally conserved in secondary structure position (i.e., are positionally homologous) in either LSU or SSU rRNA.

FORMATION OF PSEUDOURIDINE IN RNA

Pseudouridine Synthases. Ψ is synthesized at the polyribonucleotide level through the action of pseudouridine synthases, which catalyze the site-specific isomerization of U residues in RNA. Identified through a combination of biochemical and bioinformatics approaches, the pseudouridine synthases comprise a large and ancient family of proteins, with TruA, TruB, RluA, and RsuA (see below) representing four distinct subfamilies (*29*, *30*). It is possible (although not yet proven) that these enzymes arose by multiple independent gene duplications of an ancestral pseudouridine synthase.

Pseudouridine Formation in Eubacterial rRNA and in tRNA. Pseudouridine synthases exemplify two quite different mechanisms in the selection of U residues for isomerization to Ψ. In the first case, specificity derives from standard protein-based recognition of the structural context of the target U. Pseudouridine

synthases of this type include the RluA and RsuA families responsible for Ψ formation in eubacterial LSU and SSU rRNAs, respectively. In both eubacteria and eukaryotes, the TruA and TruB families use a similar system to mediate pseudouridylation in the ASL and at position 55, respectively, of tRNA. In these cases, each synthase is generally responsible for the formation of Ψ residues at one or more positions in a particular RNA species; however, dual-specificity pseudouridine synthases catalyzing the formation of Ψ residues in both tRNAs and rRNAs (*31*), tRNAs and snRNAs (*32*), and in both cytoplasmic and mitochondrial tRNAs (*33*) have been described.

Pseudouridine Formation in Eukaryotic rRNA. Whereas the relatively few Ψ residues in eubacterial rRNAs and in tRNAs are formed by site-specific pseudouridine synthases, it seemed unlikely a priori that each of the 50–100 Ψ residues in eukaryotic rRNA species (*3*, *8*) would be generated by separate pseudouridine synthases. This conundrum was resolved when a new class of small nucleolar RNAs, box H/ACA, was identified (*20*, *34*, *35*). Found (as the name implies) in the nucleolus, the site of ribosome biosynthesis in eukaryotes (*36*), the box H/ACA snoRNAs possess hairpin-hinge-hairpin-tail structures, with the hinge region containing the conserved sequence block ANANNA (box H) and the ACA motif found in the tail structure, three nucleotides from the 3′-end (*20*, *34*, *35*) (Fig. 5).

In this system, site selection for pseudouridylation occurs by transient base-pair interactions between antisense elements in the box H/ACA snoRNA and sequences in the rRNA on either side of the target U residue (Fig. 5). This complementary base-pairing creates a pseudouridylation pocket in which the target U is unpaired and thus available to its synthase (*20*, *35*). In the formation of a functional small nucleolar ribonucleoprotein (snoRNP) particle, each box H/ACA snoRNA associates

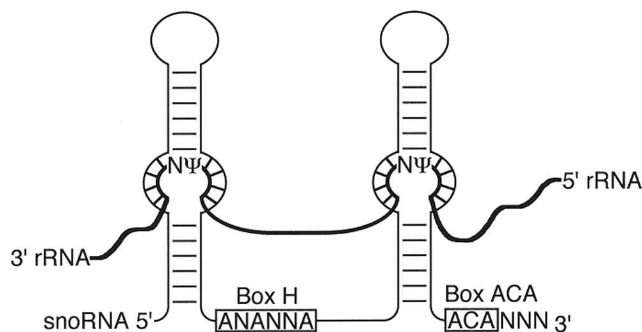

Figure 5. Schematic representation of a box H/ACA snoRNA acting on rRNA. One or both hairpins contain bulged pseudouridylation pockets that constitute short regions of complementarity to sequences flanking the target uridine (here shown as the product, Ψ) in rRNA (bold line). The only conserved sequence motifs are box H in the hinge region and box ACA, three nucleotides from the 3′ end of the snoRNA. The figure is modified from Balakin et al. (*34*).

with at least four specific proteins, one of which (Cbf5p) is a generic pseudouridine synthase. Cbf5p, originally isolated as centromere-binding factor 5 protein (*37*), is a member of the TruB family of pseudouridine synthases (*29*, *38*). This snoRNA-associated pseudouridine synthase appears to direct formation of most, if not all, of the Ψ residues in eukaryotic LSU and SSU rRNAs (*39*, *40*), with each box H/ACA snoRNA effectively guiding Cbf5p to one and occasionally two target sites (*20*, *35*). Box H/ACA snoRNAs may also mediate Ψ formation in snRNAs (e.g., U6) and snoRNAs (e.g., U3) (*19*).

Mechanism of Pseudouridine Formation. Unlike other nucleoside modification reactions, U Ψ isomerization has no known energy or coenzyme requirement (*2*), although zinc is an essential (structural?) cofactor in at least one pseudouridine synthase (*41*). Recent mutagenic (*40*, *42*, *43*), biochemical (*44*), and crystallographic (*45*) studies have identified a catalytic aspartate residue in pseudouridine synthases and provided insight into the enzymatic mechanism by which Ψ formation occurs in RNA (see Fig. 1). In the first step of this model, the universally conserved Asp (corresponding to Asp60 in *E. coli* TruA) carries out a nucleophilic attack on C-6 of the uracil ring in the target U. This breaks the N1-C1′ glycosyl bond, separating the base from its ribose partner. The freed base, attached by a C6-Oδ1 linkage to the side chain of the catalytic Asp, is rotated 180° through a diagonal N3-C6 axis (Fig. 1). The base is then reattached to the ribose by a C5-C1′ glycosyl bond and liberated from its synthase.

POSTULATED BIOLOGICAL ROLES OF PSEUDOURIDINE RESIDUES IN RNA

Transfer RNA

During translation, Ψ is thought to modulate some of the many interactions that tRNA molecules make with rRNAs and with mRNAs. Pseudouridylation of tRNA does not influence the overall three-dimensional structure of tRNAs, is not essential for cell viability, and is not generally required for aminoacylation (*13*, *16*, *46*). However, Ψ does affect the local structure of the domains in which it resides (see *6*).

The influence of modified nucleosides, including but not limited to Ψ residues, on the local structure of the ASL appears to be critical for the proper binding of tRNA to the ribosome. This effect may be mediated by a tighter binding of the appropriate tRNA to the 30S ribosomal subunit through stabilization of the dynamic structure of the anticodon loop (*11*, *14*, *47*; but see also *12*). Maintenance of the proper conformation of the three anticodon residues undoubtedly helps to foster correct codon–anticodon interactions. This may increase translational accuracy by decreasing the rate of peptide bond formation, thereby allowing more time for rejection of incorrect codon–anticodon pairs (*46*).

Ψ residues, occasionally found in the anticodon proper, play a role in alternative codon usage. In the mitochondria of echinoderms, a $tRNA^{Asn}$ having the anticodon GΨU translates the

AAA lysine codon as asparagine (*48*). Also, eukaryotic cytoplasmic $tRNA^{Tyr}$ (anticodon GΨA) is a suppressor of UAA and UAG stop codons (see *48*). In yeast, a minor $tRNA^{Ile}$ variant has the anticodon ΨAΨ (*49*). In these contexts, Ψ34, Ψ35, and Ψ36 are postulated to strengthen codon–anticodon base-pairing, particularly noncanonical interactions involving G-A or G-G wobble base pairs (*11*). Thus, pseudouridylation plays an important biological role in fine-tuning the structure of those tRNAs in which it occurs, thereby influencing their decoding activity, improving the fidelity of protein biosynthesis, and helping to maintain the proper reading frame (*46*).

Ribosomal RNA

Compared with tRNA, far less structure–function information is available for Ψ residues in rRNA. At the moment, functional inferences for Ψ in rRNA are largely limited to extrapolations from the known biochemical and biophysical effects of Ψ residues in other contexts, superimposed on our knowledge of the distribution of Ψ in individual rRNA species.

Ψ residues may influence both rRNA folding and ribosome assembly (*8*, *50*). The structural stability afforded by Ψ may assist in guiding the folding of rRNA toward a productive—or away from a dead-end—path; one possibility is that box H/ACA snoRNAs mediate rRNA folding and that pseudouridylation might then serve as a snoRNA/rRNA dissociation signal once the rRNA molecule has assumed its proper structural conformation (*8*). In the mature ribosome, Ψ probably contributes to the stabilization of local secondary or tertiary structure through RNA–RNA or RNA–protein interactions (or both) (*3*). Such fine-tuning of local higher-order structure in rRNA could influence the speed and accuracy of decoding and proofreading during translation by fostering appropriate interactions with tRNAs and by promoting catalytic efficiency in peptide bond formation (*2*). Pseudouridine residues may also stabilize the multiple contacts that are made, broken, and reformed during movement of tRNA, mRNA, and rRNA within the translating ribosome.

A catalytic role in peptidyl transfer during translation has been considered for Ψ in rRNA. This idea (*2*, *9*) was prompted by recognition of the provocative clustering of Ψ residues in the PTC of the LSU rRNA and by the intriguing possibility that the N-1 position of Ψ might exhibit a high group transfer potential for acyl moieties. However, recent observations seem to argue against a direct involvement of Ψ in the chemical mechanism of ribosome-mediated peptide bond synthesis. First, as previously noted (*2*), no Ψ residues are absolutely conserved in position in the LSU rRNA in all three domains of life, or even within a single domain (*8*); thus, any requirement for Ψ in the PTC of the ribosome cannot be universally site-specific—although reasons that might account for this lack of universality have been elaborated elsewhere (*2*). Second, genetic knockout of the snR10 gene in yeast is not lethal (*20*). This gene encodes a box H/ACA snoRNA that mediates formation of a candidate (*2*, *9*) catalytic Ψ residue in eukaryotic LSU rRNA (see Fig. 4). Third, a decrease but not complete loss of peptidyltransferase activity has been reported in experiments using 50S ribosomal subunits (*Bacillus stearothermophilus* and *Thermus aquaticus*) reconstituted with unmodified 23S rRNA transcribed in vitro (*51*, *52*). Finally, induced loss through genetic depletion experiments of most of the Ψ residues in yeast rRNA, including all of those in the PTC, is not lethal (see below).

Although the "pseudouridine hypothesis" of Lane et al. (*2*, *9*) seems at this stage to be unsupportable in its original form, there is no question that it has focused attention on and galvanized research into possible biochemical functions of Ψ in rRNA. One legacy of this hypothesis is a greatly increased knowledge of the localization and phylogenetic distribution of Ψ in rRNA (*21*, *23*, *24*) and the discovery and characterization of pseudouridine synthases and their genes (see below).

GENETIC ANALYSIS OF PSEUDOURIDINE FUNCTION IN RNA

Transfer RNA

The first genetic evidence supporting a biological role for Ψ in RNA came from studies of the phenotypic effects of the *hisT* mutation in *E. coli* and *Salmonella typhimurium*. The *hisT* gene encodes pseudouridine synthase I (PusI), a member of the TruA family and responsible for formation of Ψ at positions 38, 39, and 40 in the ASL of about half of all tRNA isoacceptors (see *53*). Transfer RNAs that lack Ψ38, Ψ39, and Ψ40 (as a result of mutations in *hisT*) are efficiently charged with their cognate amino acid but exhibit problems at the aminoacyl-tRNA selection step during translation, possibly because of the absence of the stabilizing effects of Ψ38/39/40 (*12*) on the multiple contacts ordinarily made between mRNA, rRNA, and the ASL of tRNA in the ribosomal A-site. As a result, the attenuation mechanism that regulates the transcription of many biosynthetic operons does not operate in *hisT* mutants (see *53*), leading to constitutive expression of the *his*, *leu*, *tyr*, *lys*, and *ilv* operons. In addition, *hisT* mutant strains display a 20–25% reduction in the rate of polypeptide chain elongation and exhibit pleiotropic abnormalities in cell division processes, resulting in an increase in doubling time of ~30% (*54*).

In yeast (*S. cerevisiae*), the *DEG1* gene encodes a TruA-type Ψ synthase (Deg1p = Pus3p) responsible for Ψ formation at positions 38 and 39 in cytoplasmic tRNAs. Disruption of *DEG1* results in a reduced growth rate, especially at higher temperatures (*55*). Genetic disruption of the yeast gene *PUS1*, which specifies the synthase (Pus1p) responsible for pseudouridylation at positions 34 and 36 in the anticodon proper and elsewhere in the tRNA, is without detectable phenotypic effect (*56*, *57*). Moreover, *PUS1-DEG1* double mutants do not exhibit a slow growth phenotype beyond that seen with *DEG1* mutants alone (*55*). However, simultaneous loss of function in *PUS1* and in another locus, *LOS1*, leads to reduced growth rate at 30 °C and no growth at 37 °C (*56*, *57*). The Los1p protein may function as an importin-like factor in the nuclear export of tRNAs; consequently, it has been proposed that Pus1p-catalyzed Ψ modification may be

necessary for interaction of mature tRNAs with Los1p during their export from the nucleus (*57*).

Finally, disruption of the yeast gene *PUS4*, the protein product of which (Pus4p) is responsible for formation of Ψ55, has no detectable effect on growth (*33*). Earlier work had demonstrated only a small decrease in growth rate after disruption of the homologous *E. coli* gene, TruB (see *58*). Lack of a significant phenotype in Ψ55-deficient tRNAs is surprising, considering that Ψ55 is almost universally conserved in tRNA. Nevertheless, Ψ55 is evidently not required for aminoacylation, nor is it required for translation in vitro (*33*).

Ribosomal RNA in Eubacteria

In eubacteria, the role of Ψ residues in rRNA function has been assessed by gene disruption of the corresponding Ψ synthases. Knockout of *rsuA* (responsible for formation of Ψ516, the sole Ψ residue in *E. coli* SSU rRNA [*59*]), *rluA* (Ψ746 in LSU rRNA and Ψ32 in some tRNAs [*60*]), and *rluC* (pseudouridylation of positions 955, 2504, and 2580 in the LSU rRNA [*61*, *62*]), has little or no discernible effect on exponential growth rate in either rich or minimal media at temperatures ranging from 24 °C to 42 °C. This is surprising, considering that Ψ516 in *E. coli* SSU rRNA is located in the 530 loop (*63*), which is implicated in the fidelity of codon recognition, and that two of the missing LSU rRNA Ψ residues, Ψ2504 and Ψ2580, are localized in the PTC. On the other hand, mutant phenotypes were observed for *rsuA*, *rluA*, and *rluC* knockout strains when their growth characteristics (including survival in stationary phase and length of lag phase) were assessed in competition experiments involving mixed cultures of mutant and wild-type strains (*60*). Thus, the presence of Ψ516 in SSU rRNA and of Ψ746, Ψ955, Ψ2504, and Ψ2580 in LSU rRNA appears to confer a slight but significant selective advantage on the wild-type *E. coli* strain, as seen by a gradual decrease in the proportion of *rsuA* and *rluC* mutants and a sharp decrease in the proportion of *rluA* mutants over time.

The pseudouridine synthase encoded by *E. coli rluD* is responsible for formation of Ψ1911, Ψ1915, and Ψ1917 (*62*, *64*), all of which are in mRNA-decoding domain IV of the LSU rRNA. In marked contrast to functional disruption of *rluA*, *rluB*, and *rluC*, knockout of *rluD* in *E. coli* results in severe inhibition of growth (*62*, *64*), possibly as a direct consequence of disturbances in the structure and decoding activity of domain IV. Thus, *rluD* is an essential gene for normal growth in *E. coli*.

Ribosomal RNA in Eukaryotes

In eukaryotic rRNAs, the functional role of any particular Ψ residue can be assessed by disruption of the corresponding box H/ACA snoRNA. In *S. cerevisiae*, no observable growth defect is seen after individual loss of at least 18 of the 45 experimentally verified Ψ residues in the ribosome. Deletions of multiple box H/ACA snoRNAs, resulting in simultaneous loss of five (*65*) or seven (*66*) Ψ residues, similarly had no observable effect on growth, although in neither case were competition experiments performed in the presence of the wild-type strain. The mutant strain harboring four disrupted box H/ACA snoRNA genes (specific for five Ψ sites: three in LSU rRNA and two in SSU rRNA) also displayed no discernible growth defect when cultured on different carbon sources and at temperatures between 15 °C and 37 °C, with normal cell morphologies and bud formation evident (*65*). However, in both of the above cases, the Ψ residues that are missing correspond to residues that are distributed among different rRNA domains (see Fig. 4) rather than being concentrated in a particular domain. The individual loss of all eight Ψ residues in the vicinity of the PTC in yeast (Fig. 4) resulted in no observable defects in cell viability or growth (*8*); however, the effect of simultaneous loss of these eight Ψ residues has not yet been assessed. In *S. cerevisiae*, genetic depletion of Gar1p, one of the protein components of box H/ACA snoRNPs, results in the loss of virtually all Ψ residues in the SSU and LSU rRNAs (*67*). Although such cells are viable, their growth behavior has not yet been investigated.

Global loss of Ψ residues in yeast has been studied by mutation of the box H/ACA snoRNA-associated pseudouridine synthase, Cbf5p. However, interpretation of the results of such experiments is problematic because Cbf5p forms associations with microtubules and with the kinetochore complex in chromosome segregation (*37*); with the meiosis-specific protein kinase, Mck1 (*68*); with the RNA polymerase I transcription factor, RRN3, involved in pre-rRNA transcription (*69*); with hTR, the RNA component of human telomerase (*70*); and with the box H/ACA snR30, which is involved in endonucleolytic pre-rRNA processing and the activity of which is essential (*39*). Nonetheless, genetic depletion of Cbf5p in *S. cerevisiae* has a dramatic effect, resulting in the near global abolition of Ψ in rRNAs and a gradual decrease in growth rate, with eventual arrest of cell division at the G_1/S boundary and lethality (*37*, *39*). Point mutations introduced into the catalytic domain of Cbf5p yield cells that are viable at 25 °C but display cold- and heat-sensitive growth phenotypes and reduced Ψ content in rRNA (*40*). Substitution of the catalytic Asp by Ala globally abolishes Ψ formation in rRNA under all temperature conditions. Mutant strains carrying this substitution are viable but exhibit severe growth defects at temperatures below 30 °C and no growth at 37 °C. However, this mutation may also influence the interaction of Cbf5p with the box H/ACA snoRNAs as well as rRNA stability, as suggested by decreased levels of snoRNPs and both 18S and 25S rRNAs (*40*).

In *Drosophila*, the Cbf5p ortholog Nop60B (*71*), also known as *minifly* (*72*), is essential for viability and fertility. P-element–induced deletions of the entire Nop60B ORF lead to homozygous lethality late in development (*71*, *72*). Partial loss-of-function mutations in Nop60B have dramatic effects during embryogenesis and in subsequent development, with morphological abnormalities evident in certain tissues (*72*). Examination of rRNA in mutant flies revealed inefficient pre-rRNA processing and decreased pseudouridylation in both 28S and 18S rRNAs (*72*).

A HUMAN GENETIC DISORDER ASSOCIATED WITH MUTATIONS IN A PSEUDOURIDINE SYNTHASE

Mutations in the human gene encoding dyskerin, a Cbf5p ortholog, have been associated with the rare haematopoietic and malignant disorder, X-linked dyskeratosis congenita (*73*, *74*). Initially, a defect in rRNA processing or maturation (Ψ-deficient ribosomes?) was thought to be the fundamental cause of this disorder; however, normal amounts of Ψ and mature rRNAs have since been found in the ribosomes of affected individuals (*70*). Instead, cells of individuals with this disorder have a lower content of hTR; consequently, telomerase activity is decreased, with a resulting difficulty in maintaining telomere length (*70*). Interestingly, the 3′-terminal region of mammalian hTR has the characteristic primary sequence blocks and secondary structure features of a box H/ACA snoRNA (*75*); thus, dyskerin may associate with this region. Whether there is a direct link between the pseudouridylation function of dyskerin, hTR, and X-linked dyskeratosis congenita remains to be seen.

CONCLUSIONS

Although Ψ has been slow to give up the secrets of its roles in RNA, many of the "where" and "how" questions have yielded to the combination of biochemical, biophysical, and genetic approaches summarized here. The "why" questions, however, remain only partially answered. Considering that Ψ is so abundant and so widely distributed in naturally occurring RNAs, and that so much genetic information (in the form of Ψ synthases and snoRNAs) is invested in its formation, it is a reasonable conjecture that this modified nucleoside is an important, even essential, constituent of RNA *in the biological contexts in which it normally exists*. The profound phenotypic effects seen in some cases of Ψ deficiency in RNA (*40*, *54*, *64*) fully support this view. Particularly telling in this regard are those genetic experiments in which loss of one or more Ψ residues has little or no discernible effect on growth and other measurable parameters, yet the mutant strain is unable to compete effectively with the parent strain in mixed-culture experiments. Given the apparent subtlety of much of the "Ψ effect" in RNA, standard biochemical and genetic approaches in a laboratory setting may provide only limited insight into Ψ function, especially with regard to the importance of any given Ψ residue in an individual RNA species.

The consensus view is that most Ψ residues are likely to serve a structural role (acting as "structural struts" [see *24*]) in RNA. More than 30 years ago, McLennan and Lane (*76*) inferred that Ψ residues are probably concentrated at the junctions between single-stranded and helical regions in tRNA and rRNA. Detailed physicochemical studies (*5*, *6*) have now elucidated the consequences of such localization, since confirmed, in stabilizing higher-order structures.

Nevertheless, to conclude that all Ψ residues occupy similar environments in all of the RNA molecules in which they occur, and play the same role at all positions at which they are located, would be premature. Just as a given amino acid (e.g., Asp) may serve either a structural or a catalytic role in a protein, so too might a given modified nucleoside (e.g., Ψ) have different functions depending on the local environment in which it occurs. In eukaryotic LSU rRNA, in particular, the curious coclustering of Ψ and $O^{2'}$-methylnucleoside residues (*3*, *24*), as well as the presence of divalent metal ions (particularly Mg^{2+}), could conceivably create local environments that alter the function of selected Ψ residues (see *2*).

As a result of findings (*64*) stimulated by the "pseudouridine hypothesis" of Lane et al. (*9*), the focus for a possible translational role for Ψ has now shifted from domain V (PTC) to domain IV (mRNA decoding site). In this regard, it would be very interesting to determine the phenotypic effect in yeast of the simultaneous loss of the same Ψ residues that are absent from domain IV in the LSU rRNA of the *E. coli rluD* mutant. With respect to a possible catalytic role for Ψ in LSU rRNA (*9*), it is notable that reconstituted 50S ribosomal subunits containing modification-free 23S rRNA are able to carry out peptide bond synthesis, albeit with substantially lower efficiency than 50S subunits reconstituted with the corresponding natural rRNA species (*51*, *52*). This finding would seem to preclude the type of role originally envisaged (*9*) for Ψ in the chemistry of peptide bond synthesis. However, in assessing the role of modified nucleosides in rRNA function, we would do well to remember that such experiments are still a long way from the "real" biological situation, where the translating ribosome, in decoding natural mRNA, catalyzes multiple rounds of peptide bond formation with exquisite accuracy and efficiency.

Biophysical studies of Ψ-containing tRNAs have been especially revealing about the structural consequences of Ψ in this RNA species. We can look forward to comparable insights for Ψ in rRNA and snRNA, while recognizing that the corresponding studies, particularly in the context of a ribosomal particle or splicing complex, are likely to be orders of magnitude more difficult. In this regard, naturally fragmented rRNAs, in which rRNA domains and subdomains are distributed among separate RNA species (e.g., *77*), may ultimately prove useful. Although genetic experiments in yeast, based on snoRNA gene ablation, have yielded the somewhat surprising result that loss of multiple Ψ residues from an rRNA molecule is not necessarily lethal (or even obviously detrimental), additional studies of this sort are needed to assess the consequences of simultaneous loss of all of the Ψ residues within functionally important domains (e.g., the peptidyltransferase and mRNA decoding centers of LSU rRNA). Another approach to elucidating Ψ function in rRNA (or snRNA) might be to target artificial snoRNA constructs to U residues that are not ordinarily pseudouridylated, and to assess the biophysical and other parameters of such supramodified RNAs in comparison with their normally modified counterparts. Such an approach has been used successfully to alter the $O^{2'}$–methylation status of rRNA (*78*). Finally, other aspects of RNA structure and function that have been addressed elsewhere, such as the importance of modified nucleosides in coordinating metal ions (particularly Mg^{2+}) (*4*) and the

provocative clustering of Ψ and $O^{2'}$-methylnucleoside residues in LSU rRNA (*3*), should continue to be explored.

ACKNOWLEDGEMENTS

We are indebted to Prof. B. G. Lane, University of Toronto, for providing many pertinent insights into the development of this area of research and for cogent comment on the manuscript. We are also grateful to members of the Gray lab (particularly Drs. M. N. Schnare, D. F. Spencer, A. Lohan, and Y. Watanabe) for their valuable critique and for assistance in preparing figures. We thank Dr. C. H. Schwab (Universität Erlangen-Nürnberg) for making available coordinates for the water molecule in Fig. 2 and Dr. R. R. Gutell (University of Texas at Austin) for supplying electronic files of the secondary structure used in Fig. 4. Work in the authors' laboratory on modification and processing of eukaryotic rRNA is supported by an operating grant (MT-12112 to M.W.G.) and studentship (to M.C.) from the Medical Research Council of Canada. Salary and interaction support in the form of a fellowship to M.W.G. from the Canadian Institute for Advanced Research (Program in Evolutionary Biology) is gratefully acknowledged.

NOTE ADDED IN PROOF

A newly identified pseudouridine synthase, Pus5p, mediates formation of the lone Ψ in yeast (*S. cerevisiae*) mitochondrial LSU rRNA, at a position (2819) corresponding to Ψ2580 in *E. coli* 23S rRNA [Ansmant, I., Massenet, S., Grosjean, H., Motorin, Y., and Branlant, C. (2000) Identification of the *Saccharomyces cerevisiae* RNA:pseudouridine synthase responsible for formation of Ψ_{2819} in 21S mitochondrial ribosomal RNA. *Nucleic Acids Res.* **28**, 1941–1946]. As observed when the analogous *E. coli* gene (*rluC*) was disrupted, a yeast *PUS5* knockout strain did not display any apparent growth defect when cultured on rich or minimal medium at temperatures of 20 °, 30 °, or 37 ° C. Thus, viability of *S. cerevisiae* is not compromised even when its mitochondrial ribosomes totally lack Ψ residues.

In a phylogenetic comparison of deduced secondary structure, a 3′-terminal box H/ACA snoRNA motif was found to be characteristic of vertebrate but not ciliate telomerase RNAs [Chen, J.-L., Blasco, M. A., and Greider, C. W. (2000) Secondary structure of vertebrate telomerase RNA. *Cell* **100**, 503–514]. However, it would appear that this particular box H/ACA motif does not function in pseudouridylation in the cell.

REFERENCES

1. Lane, B. G. (1998) Historical perspectives on RNA nucleoside modifications. In *Modification and Editing of RNA* (Grosjean, H., and Benne, R., eds.). pp. 1–20, ASM Press, Washington, DC.
2. Lane, B. G., Ofengand, J., and Gray, M. W. (1995) Pseudouridine and $O^{2'}$-methylated nucleosides. Significance of their selective occurrence in rRNA domains that function in ribosome-catalyzed synthesis of the peptide bonds in proteins. *Biochimie* **77**, 7–15.
3. Ofengand, J., Bakin, A., Wrzesinski, J., Nurse, K., and Lane, B. G. (1995) The pseudouridine residues of ribosomal RNA. *Biochem. Cell Biol.* **73**, 915–924.
4. Agris, P. F. (1996) The importance of being modified: Roles of modified nucleosides and Mg^{2+} in RNA structure and function. *Prog. Nucleic Acid Res. Mol. Biol.* **53**, 79–129.
5. Davis, D. R. (1998) Biophysical and conformational properties of modified nucleosides in RNA (nuclear magnetic resonance studies). In *Modification and Editing of RNA* (Grosjean, H., and Benne, R., eds.). pp. 85–102, ASM Press, Washington, DC.
6. Auffinger, P., and Westhof, E. (1998) Effects of pseudouridylation on tRNA hydration and dynamics: A theoretical approach. In *Modification and Editing of RNA* (Grosjean, H., and Benne, R., eds.). pp. 103–112, ASM Press, Washington, DC.
7. Massenet, S., Mougin, A., and Branlant, C. (1998) Posttranscriptional modifications in the U small nuclear RNAs. In *Modification and Editing of RNA* (Grosjean, H., and Benne, R., eds.). pp. 201–227, ASM Press, Washington, DC.
8. Ofengand, J., and Fournier, M. J. (1998) The pseudouridine residues of rRNA: Number, location, biosynthesis, and function. In *Modification and Editing of RNA* (Grosjean, H., and Benne, R., eds.). pp. 229–253, ASM Press, Washington, DC.
9. Lane, B. G., Ofengand, J., and Gray, M. W. (1992) Pseudouridine in the large-subunit (23 S-like) ribosomal RNA. The site of peptidyl transfer in the ribosome? *FEBS Lett.* **302**, 1–4.
10. Neumann, J. M., Bernassau, J. M., Guéron, M., and Tran-Dinh, S. (1980) Comparative conformations of uridine and pseudouridine and their derivatives. *Eur. J. Biochem.* **108**, 457–463.
11. Davis, D. R., Veltri, C. A., and Nielsen, L. (1998) An RNA model system for investigation of pseudouridine stabilization of the codon-anticodon interaction in $tRNA^{Lys}$, $tRNA^{His}$ and $tRNA^{Tyr}$. *J. Biomol. Struct. Dyn.* **15**, 1121–1132.
12. Yarian, C. S., Basti, M. M., Cain, R. J., Ansari, G., Guenther, R. H., Sochacka, E., Czerwinska, G., Malkiewics, A., and Agris, P. F. (1999) Structural and functional roles of the N1- and N3-protons of Ψ at tRNA's position 39. *Nucleic Acids Res.* **27**, 3543–3549.
13. Arnez, J. G., and Steitz, T. A. (1994) Crystal structure of unmodified $tRNA^{Gln}$ complexed with glutaminyl-tRNA synthetase and ATP suggests a possible role for pseudo-uridines in stabilization of RNA structure. *Biochemistry* **33**, 7560–7567.
14. Durant, P. C., and Davis, D. R. (1999) Stabilization of the anticodon stem-loop of $tRNA^{Lys,3}$ by an A^+-C base-pair and by pseudouridine. *J. Mol. Biol.* **285**, 115–131.
15. Davis, D. R. (1995) Stabilization of RNA stacking by pseudouridine. *Nucleic Acids Res.* **23**, 5020–5026.
16. Davis, D. R., and Poulter, C. D. (1991) ^{1}H-^{15}N NMR studies of *Escherichia coli* $tRNA^{Phe}$ from *hisT* mutants: A structural role for pseudouridine. *Biochemistry* **30**, 4223–4231.
17. Massenet, S., and Branlant, C. (1999) A limited number of pseudouridine residues in the human atac spliceosomal UsnRNAs as compared to human major spliceosomal UsnRNAs. *RNA* **5**, 1495–1503.
18. Greenwood, S. J., Schnare, M. N., and Gray, M. W. (1996) Molecular characterization of U3 small nucleolar RNA from the early diverging protist, *Euglena gracilis*. *Curr. Genet.* **30**, 338–346.
19. Ganot, P., Jády, B. E., Bortolin, M.-L., Darzacq, X., and Kiss, T. (1999) Nucleolar factors direct the 2′-*O*-ribose methylation and pseudouridylation of U6 spliceosomal RNA. *Mol. Cell. Biol.* **19**, 6906–6917.
20. Ni, J., Tien, A. L., and Fournier, M. J. (1997) Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell* **89**, 565–573.
21. Ofengand, J., and Bakin, A. (1997) Mapping to nucleotide resolution of pseudouridine residues in large subunit ribosomal RNAs from representative eukaryotes, prokaryotes, archaebacteria, mitochondria, and chloroplasts. *J. Mol. Biol.* **266**, 246–268.

22. Szymanski, M., Barciszewska, M. Z., Barciszewski, J., and Erdmann, V. A. (2000) 5S ribosomal RNA database Y2K. *Nucleic Acids Res.* **28**, 166–167.
23. Bakin, A., and Ofengand, J. (1993) Four newly located pseudouridylate residues in *Escherichia coli* 23S ribosomal RNA are all at the peptidyltransferase center: Analysis by the application of a new sequencing technique. *Biochemistry* **32**, 9754–9762.
24. Bakin, A., Lane, B. G., and Ofengand, J. (1994) Clustering of pseudouridine residues around the peptidyltransferase center of yeast cytoplasmic and mitochondrial ribosomes. *Biochemistry* **32**, 13475–13483.
25. Brimacombe, R., Mitchell, P., Osswald, M., Stade, K., and Bochkariov, D. (1993) Clustering of modified nucleotides at the functional center of bacterial ribosomal RNA. *FASEB J.* **7**, 161–167.
26. Moazed, D., and Noller, H. F. (1989) Interaction of tRNA with 23S rRNA in the ribosomal A, P, and E sites. *Cell* **57**, 585–597.
27. Green, R., Switzer, C., and Noller, H. F. (1998) Ribosome-catalyzed peptide-bond formation with an A-site substrate covalently linked to 23S ribosomal RNA. *Science* **280**, 286–289.
28. Massenet, S., Ansmant, I., Motorin, Y., and Branlant, C. (1999) The first determination of pseudouridine residues in 23S ribosomal RNA from hyperthermophilic *Archaea Sulfolobus acidocaldarius*. *FEBS Lett.* **462**, 94–100.
29. Koonin, E. V. (1996) Pseudouridine synthases: Four families of enzymes containing a putative uridine-binding motif also conserved in dUTPases and dCTP deaminases. *Nucleic Acids Res.* **24**, 2411–2415.
30. Gustafsson, C., Reid, R., Greene, P. J., and Santi, D. V. (1996) Identification of new RNA modifying enzymes by iterative genome search using known modifying enzymes as probes. *Nucleic Acids Res.* **24**, 3756–3762.
31. Wrzesinski, J., Nurse, K., Bakin, A., Lane, B. G., and Ofengand, J. (1995) A dual-specificity pseudouridine synthase: An *Escherichia coli* synthase purified and cloned on the basis of its specificity for Ψ746 in 23S RNA is also specific to Ψ32 in $tRNA^{phe}$. *RNA* **1**, 437–448.
32. Massenet, S., Motorin, Y., Lafontaine, D. L. J., Hurt, E. C., Grosjean, H., and Branlant, C. (1999) Pseudouridine mapping in the *Saccharomyces cerevisiae* spliceosomal U small nuclear RNAs (snRNAs) reveals that pseudouridine synthase Pus1p exhibits a dual substrate specificity for U2 snRNA and tRNA. *Mol. Cell. Biol.* **19**, 2142–2154.
33. Becker, H. F., Motorin, Y., Planta, R. J., and Grosjean, H. (1997) The yeast gene YNL292w encodes a pseudouridine synthase (Pus4) catalyzing the formation of Ψ_{55} in both mitochondrial and cytoplasmic tRNAs. *Nucleic Acids Res.* **25**, 4493–4499.
34. Balakin, A. G., Smith, L., and Fournier, M. J. (1996) The RNA world of the nucleolus: Two major families of small RNAs defined by different box elements with related functions. *Cell* **86**, 823–834.
35. Ganot, P., Bortolin, M.-L., and Kiss, T. (1997) Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell* **89**, 799–809.
36. Sollner-Webb, B., Tycowski, K. T., and Steitz, J. A. (1996) Ribosomal RNA processing in eukaryotes. In *Ribosomal RNA. Structure, Evolution, Processing, and Function in Protein Biosynthesis* (Zimmermann, R. A., and Dahlberg, A. E., eds.). pp. 469–490, CRC Press, Boca Raton, FL.
37. Jiang, W., Middleton, K., Yoon, H.-J., Fouquet, C., and Carbon, J. (1993) An essential yeast protein, CBF5p, binds in vitro to centromeres and microtubules. *Mol. Cell. Biol.* **13**, 4884–4893.
38. Aravind, L., and Koonin, E. V. (1999) Novel predicted RNA-binding domains associated with the translation machinery. *J. Mol. Evol.* **48**, 291–302.
39. Lafontaine, D. L. J., Bousquet-Antonelli, C., Henry, Y., Caizergues-Ferrer, M., and Tollervey, D. (1998) The box H + ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase. *Genes Dev.* **12**, 527–537.
40. Zebarjadian, Y., King, T., Fournier, M. J., Clarke, L., and Carbon, J. (1999) Point mutations in yeast *CBF5* can abolish in vivo pseudouridylation of rRNA. *Mol. Cell. Biol.* **19**, 7461–7472.
41. Arluison, V., Hountondji, C., Robert, B., and Grosjean, H. (1998) Transfer RNA-pseudouridine synthetase Pus1 of *Saccharomyces cerevisiae* contains one atom of zinc essential for its native conformation and tRNA recognition. *Biochemistry* **37**, 7268–7276.
42. Huang, L., Pookanjanatavip, M., Gu, X., and Santi, D. V. (1998) A conserved asparate of tRNA pseudouridine synthase is essential for activity and a probable nucleophilic catalyst. *Biochemistry* **37**, 344–351.
43. Ramamurthy, V., Swann, S. L., Paulson, J. L., Spedaliere, C. J., and Mueller, E. G. (1999) Critical aspartic acid residues in pseudouridine synthases. *J. Biol. Chem.* **274**, 22225–22230.
44. Gu, X., Liu, Y., and Santi, D. V. (1999) The mechanism of pseudouridine synthase I as deduced from its interaction with 5-fluorouracil-tRNA. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14270–14275.
45. Foster, P. G., Huang, L., Santi, D. V., and Stroud, R. M. (2000) The structural basis for tRNA recognition and pseudouridine formation by pseudouridine synthase I. *Nat. Struct. Biol.* **7**, 23–27.
46. Harrington, K. M., Nazarenko, I. A., Dix, D. B., Thompson, R. C., and Uhlenbeck, O. C. (1993) In vitro analysis of translation rate and accuracy with an unmodified tRNA. *Biochemistry* **32**, 7617–7622.
47. Dao, V. Guenther, R., Malkiewicz, A., Nawrot, B., Sochacka, E., Kraszewski, A., Jankowska, J., Everett, K., and Agris, P. F. (1994) Ribosome binding of DNA analogs of tRNA requires base modifications and supports the "extended anticodon". *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2125–2129.
48. Tomita, K., Ueda, T., and Watanabe, K. (1999) The presence of pseudouridine in the anticodon alters the genetic code: A possible mechanism for assignment of the AAA lysine codon as asparagine in echinoderm mitochondria. *Nucleic Acids Res.* **27**, 1683–1689.
49. Senger, B., Auxilien, S., Englisch, U., Cramer, F., and Fasiolo, F. (1997) The modified wobble base inosine in yeast $tRNA^{Ile}$ is a positive determinant for aminoacylation by isoleucyl-tRNA synthetase. *Biochemistry* **36**, 8269–8275.
50. Cunningham, P. R., Richard, R. B., Weitzmann, C. J., Nurse, K., and Ofengand, J. (1991) The absence of modified nucleotides affects both *in vitro* assembly and *in vitro* function of the 30S ribosomal subunit of *Escherichia coli*. *Biochimie* **73**, 789–796.
51. Green, R., and Noller, H. F. (1999) Reconstitution of functional 50S ribosomes from in vitro transcripts of *Bacillus stearothermophilus* 23S rRNA. *Biochemistry* **38**, 1772–1779.
52. Khaitovich, P., Tenson, T., Kloss, P., and Mankin, A. S. (1999) Reconstitution of functionally active *Thermus aquaticus* large ribosomal subunits with in vitro-transcribed rRNA. *Biochemistry* **38**, 1780–1788.
53. Winkler, M. E. (1996) Biosynthesis of histidine. In *Escherichia coli and Salmonella Cellular and Molecular Biology*, Vol. 1, 2nd ed. (Neidhardt, F. C., ed.). pp. 485–505, ASM Press, Washington, DC.
54. Tsui, H. C. T., Arps, P. J., Connolly, D. M., and Winkler, M. E. (1991) Absence of *his*T-mediated tRNA pseudouridylation results in a uracil requirement that interferes with *Escherichia coli* K12 cell division. *J. Bacteriol.* **173**, 7395–7400.
55. Lecointe, F., Simos, G., Sauer, A., Hurt, E. C., Motorin, Y., and Grosjean, H. (1998) Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of Ψ_{38} and Ψ_{39} in tRNA anticodon loop. *J. Biol. Chem.* **273**, 1316–1323.
56. Simos, G., Tekotte, H., Grosjean, H., Segref, A., Sharma, K., Tollervey, D., and Hurt, E. C. (1996) Nuclear pore proteins are involved in the biogenesis of functional tRNA. *EMBO J.* **15**, 2270–2284.
57. Motorin, Y., Keith, G., Simon, C., Foiret, D., Simos, G., Hurt, E., and Grosjean, H. (1998) The yeast tRNA:pseudouridine synthase Pus1p displays a multisite substrate specificity. *RNA* **4**, 856–869.
58. Sands, J. F., Regnier, P., Cummings, H. S., Grunberg-Manago, M., and Hershey, J. W. B. (1988) The existence of two genes between *infB* and *rpsO* in the *Escherichia coli* genome: DNA sequencing and S1 nuclease mapping. *Nucleic Acids Res.* **22**, 10803–10816.
59. Conrad, J., Niu, L., Rudd, K., Lane, B. G., and Ofengand, J. (1999) 16S ribosomal RNA pseudouridine synthase RsuA of *Escherichia coli*: Deletion, mutation of the conserved Asp102 residue, and sequence comparison among all other pseudouridine synthases. *RNA* **5**, 751–763.
60. Raychaudhuri, S., Niu, L., Conrad, J., Lane, B. G., and Ofengand, J. (1999) Functional effect of deletion and mutation of the *Escherichia coli* ribosomal

RNA and tRNA pseudouridine synthase RluA. *J. Biol. Chem.* **274**, 18880–18886.

61. Conrad, J., Sun, D., Englund, N., and Ofengand, J. (1998) The *rluC* gene of *Escherichia coli* codes for a pseudouridine synthase that is solely responsible for synthesis of pseudouridine at positions 955, 2504, and 2580 in 23 S ribosomal RNA. *J. Biol. Chem.* **273**, 18562–18566.
62. Huang, L., Ku, J., Pookanjanatavip, M., Gu, X., Wang, D., Greene, P. J., and Santi, D. V. (1998) Identification of two *Escherichia coli* pseudouridine synthases that show multisite specificity for 23S RNA. *Biochemistry* **37**, 15951–15957.
63. Bakin, A., Kowalak, J. A., McCloskey, J. A., and Ofengand, J. (1994) The single pseudouridine residue in *Escherichia coli* 16S RNA is located at position 516. *Nucleic Acids Res.* **22**, 3681–3684.
64. Raychaudhuri, S., Conrad, J., Hall, B. G., and Ofengand, J. (1998) A pseudouridine synthase required for the formation of two universally conserved pseudouridines in ribosomal RNA is essential for normal growth of *Escherichia coli. RNA* **4**, 1407–1417.
65. Samarsky, D. A., Balakin, A. G., and Fournier, M. J. (1995) Characterization of three new snRNAs from *Saccharomyces cerevisiae*: snR34, snR35 and snR36. *Nucleic Acids Res.* **23**, 2548–2554.
66. Parker, R., Simmons, T., Shuster, E. O., Siliciano, P. G., and Guthrie, C. (1988) Genetic analysis of small nuclear RNAs in *Saccharomyces cerevisiae*: Viable sextuple mutant. *Mol. Cell. Biol.* **8**, 3150–3159.
67. Bousquet-Antonelli, C., Henry, Y., Gélugne, J.-P., Caizergues-Ferrer, M., and Kiss, T. (1997) A small nucleolar RNP protein is required for pseudouridylation of eukaryotic ribosomal RNAs. *EMBO J.* **16**, 4770–4776.
68. Jiang, W., and Koltin, Y. (1996) Two-hybrid interaction of a human UBC9 homolog with centromere proteins of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **251**, 153–160.
69. Cadwell, C., Yoon, H.-J., Zebarjadian, Y., and Carbon, J. (1997) The yeast nucleolar protein Cbf5p is involved in rRNA biosynthesis and interacts genetically with the RNA polymerase I transcription factor *RRN3. Mol. Cell. Biol.* **17**, 6175–6183.
70. Mitchell, J. R., Wood, E., and Collins, K. (1999) A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* **402**, 551–555.
71. Phillips, B., Billin, A. N., Cadwell, C., Buchholz, R., Erickson, C., Merriam, J. R., Carbon, J., and Poole, S. J. (1998) The *Nop60B* gene of *Drosophila* encodes an essential nucleolar protein that functions in yeast. *Mol. Gen. Genet* **260**, 20–29.
72. Giordano, E., Peluso, I., Senger, S., and Furia, M. (1999) *minifly*, a *Drosophila* gene required for ribosome biogenesis. *J. Cell. Biol.* **144**, 1123–1133.
73. Heiss, N. S., Knight, S. W., Vulliamy, T. J., Klauck, S. M., Wiemann, S., Mason, P. J., Poustka, A., and Dokal, I. (1998) X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat. Genet.* **19**, 32–38.
74. Knight, S. W., Heiss, N. S., Vulliamy, T. J., Greschner, S., Stavrides, G., Pai, G. S., Lestringant, G., Varma, N., Mason, P. J., Dokal, I., and Poustka, A. (1999) X-linked dyskeratosis congenita is predominantly caused by missense mutations in the DKC1 gene. *Am. J. Hum. Genet.* **65**, 50–58.
75. Mitchell, J. R., Cheng, J., and Collins, K. (1999) A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3′ end. *Mol. Cell. Biol.* **19**, 567–576.
76. McLennan, B. D., and Lane, B. G. (1968) The chain termini of polynucleotides formed by limited enzymic fragmentation of wheat embryo ribosomal RNA. Part I. Studies of snake venom phosphodiesterase. *Can. J. Biochem.* **46**, 81–92.
77. Schnare, M. N., and Gray, M. W. (1990) Sixteen discrete RNA components in the cytoplasmic ribosome of *Euglena gracilis*. *J. Mol. Biol.* **215**, 73–83.
78. Cavaille, J., Nicoloso, M., and Bachellerie, J. P. (1996) Targeted ribose methylation of RNA *in vivo* directed by tailored antisense RNA guides. *Nature* **383**, 732–735.
79. Sadowski, J., Gasteiger, J., and Klebe, G. (1994) Comparison of automatic three-dimensional model builders using 639 X-ray structures. *J. Chem. Inf. Comput. Sci.* **34**, 1000–1008.
80. Sayle, R. A., and Milner-White, E. J. (1995) RASMOL: Biomolecular graphics for all. *Trends Biochem. Sci.* **20**, 374–376.
81. Rubin, G. M. (1973) The nucleotide sequence of *Saccharomyces cerevisiae* 5.8S ribosomal ribonucleic acid. *J. Biol. Chem.* **248**, 3860–3875.
82. Schnare, M. N., Damberger, S. H., Gray, M. W., and Gutell, R. R. (1996) Comprehensive comparison of structural characteristics in eukaryotic cytoplasmic large subunit (23 S-like) ribosomal RNA. *J. Mol. Biol.* **256**, 701–719.