METHOD

Enhanced detection of tRNA isoacceptors by combinatorial oligonucleotide hybridization

ADA BUVOLI, MASSIMO BUVOLI, and LESLIE A. LEINWAND

Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder,
Boulder, Colorado 80309, USA

ABSTRACT

A method that greatly enhances the detection of tRNA by oligodeoxyribonucleotide probe hybridization has been developed. Because highly structured tRNA regions often preclude heteroduplex formation, we have tested the ability of cold oligodeoxyribonucleotides called *unfolding* to disrupt the tRNA secondary/tertiary structures and promote hybridization of a second labeled oligonucleotide complementary to the anticodon loop. Here we show that an excess of *unfolding* in the pre/hybridization reaction can enhance a barely detectable hybridization signal by more than 200-fold without affecting probe specificity. This sensitive assay makes it possible to easily study and monitor changes in tRNA isoacceptor expression.

Keywords: isoacceptors; oligodeoxyribonucleotide hybridization; tRNA secondary/tertiary structure; *unfolding*

INTRODUCTION

Numerous observations suggest that transfer RNA, although primarily involved in amino acid activation, may also control gene expression at different levels (reviewed in Söll, 1993). For instance, bacterial development can be regulated by low-abundance tRNA isoacceptors that modulate the expression of genes containing matching rare codons (Saier, 1995). Proteolytic degradation of several polypeptides can be triggered by a tRNA-dependent posttranslational addition of amino acids to the amino terminal residues of the protein. This represents another example of the physiological versatility of the tRNA (Varshavsky, 1996).

Regulation of the expression of specific tRNAs has been reported in response to cellular differentiation and growing conditions (reviewed in Sprague, 1995). However, different pathological conditions have also been associated with changes in tRNA metabolism. Although point mutations in mitochondrial tRNA genes have been linked to numerous neuromuscular diseases (Graeber & Muller, 1998), variations in tRNA modifications (Dirheimer et al., 1995) or tRNA expression (Mukerjee &

Goldfeder, 1976; White, 1997) are often found in conjunction with neoplastic cell proliferation.

In a mammalian cell, there are probably more than 150 tRNA species generated by at least 1,300 tRNA genes (Hatlen & Attardi, 1971; Söll, 1993). Thus, the identification of individual isoacceptors by Northern blot analysis can be an initial approach to study tRNA involvement in different cellular processes. However, the use of this approach is limited by the tRNA secondary/tertiary structures that make the hybridization efficiency of specific oligonucleotide probes unpredictable (Kumazawa et al., 1992; Mir & Southern, 1999; Petyuk et al., 1999).

Here we show that a combination of oligodeoxynucleotides employed in the pre/hybridization reaction can reshape the tRNA structure and increase the sensitivity of specific detection of a suppressor tRNA derived from the human serine tRNA up to 220-fold. By removing this hindrance to tRNA hybridization, this approach greatly improves the sensitivity of Northern blot analysis so that identification of individual tRNA isoacceptors expressed at low levels can be undertaken.

RESULTS AND DISCUSSION

In a previous study, we multimerized the human serine suppressor tRNA gene on a single plasmid and assessed the ability of tandemly repeated tRNA genes to

Reprint requests to: Leslie A. Leinwand, Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder, Boulder, Colorado 80309-0347, USA; e-mail: Leslie.Leinwand@Colorado.edu.

suppress premature stop codons (Buvoli et al., 2000). To determine the expression of different multimerized suppressor tRNA constructs, we first tested the ability of two oligodeoxynucleotide probes (oligonucleotides U and α ; Fig. 2) to discriminate between the suppressor (tRNA^{su+}) and the endogenous serine tRNA (Fig. 1). In a preliminary set of experiments designed to determine the optimal oligonucleotide hybridization conditions, we found that both oligonucleotides hybridized specifically but very poorly to the tRNA^{su+}. In contrast, they hybridized efficiently to a DNA plasmid carrying the same gene (data not shown).

Based on these results and the observation that oligonucleotide probes can invade tRNA hairpin structures if a short single-stranded sequence is available to initiate the strand-displacement process (Petyuk et al., 1999), we next studied the ability of longer probes (U1, α 1, and α 2; Fig. 2) to disrupt the secondary structure of the anticodon, maintaining at the same time specificity for the tRNA^{su+}. Because the D arm is predicted to be the less stable tRNA structure (Fig. 3; Crothers et al., 1974), an oligonucleotide

FIGURE 1. Sequence and predicted cloverleaf structure of human serine suppressor tRNA (tRNA^{su+}) obtained by direct mutagenesis of human tRNA^{ser} (Capone et al., 1985). Numbering system of nucleotides is according to Sprinzl et al. (1980). The circled U at position 35 indicates the only nucleotide change introduced to create the suppressor (G → U). Modified nucleotides are reported according to Capone et al. (1985) and Buvoli et al. (2000). Tertiary interactions are identified by lines (Heckl et al., 1998).

complementary to this region was used as a reference for hybridization efficiency (oligonucleotide D3; Fig. 2). The results of this analysis performed on tRNA^{su+} made in mammalian cells showed that although the efficiency of hybridization can be increased with longer probes derived from oligonucleotide α (up to ~26-fold using oligonucleotide α 2), specificity was lost despite high stringent wash conditions (Fig. 2). Oligonucleotide D3 hybridized to the tRNA^{su+} much more efficiently than the rest of the oligonucleotides complementary to the anticodon loop, confirming that this region of the tRNA seems to be more available for hybridization (Kumazawa et al., 1992; Mir & Southern, 1999).

To get around this physical/chemical constraint, we next attempted to selectively disrupt the tRNA^{su+} intramolecular base pairing as well as other stacking interactions responsible for the tRNA tertiary structure. To this aim, we designed an array of oligodeoxynucleotides targeting different tRNA^{su+} regions (Fig. 3), and we tested their ability to unfold the tRNA structure and enhance the hybridization of oligonucleotide α . According to their potential activity, these oligonucleotides were called unfolders and were employed in the pre/hybridization reaction as described in Material and methods.

The observations that in vitro transcribed human serine tRNA is a good substrate for aminoacylation (Achse & Gross, 1993; Heckl et al., 1998) suggests that, after transcription, the tRNA should fold correctly. Based on this assumption, we also studied the interference of modified nucleotides on the formation of base–base hydrogen bonding (Kumazawa et al., 1992) by comparing the hybridization efficiency of a tRNA^{su+} made in mammalian cells with an unmodified in vitro transcribed tRNA^{su+}.

Figure 4A shows the results of such analysis obtained using single unfolders in combination with the 5'-end-labeled oligonucleotide α . When tested alone, the barely detectable hybridization signal of oligonucleotide α was threefold higher on in vitro-transcribed tRNA^{su+}, despite the different amount of suppressor tRNA loaded on the gel (2 ng of in vitro-transcribed tRNA versus ~28 ng of in vivo-made tRNA^{su+}). This result clearly shows that the presence of modifications in the tRNA^{su+} anticodon arm (Fig. 1) dramatically reduces the hybridization efficiency of small oligonucleotide probes. However, the signal did not significantly improve when unfolders targeting the acceptor arm, the T Ψ C loop and the extra arm (T1, T2, and X respectively) were employed. In contrast, ~15- and ~43-fold increases were detected on in vitro and in vivo tRNA^{su+}, respectively, with unfolders X1, that overlaps 5 nt of the anticodon stem. When the enhancing activity of the longer 40-mer TX unfolders was investigated, we discovered that the hybridization signal of oligonucleotide α increased up to 20-fold for the in vitro-transcribed and 100-fold for the in vivo-made tRNA^{su+}.

Oligo	Sequence (5'→3')	Length	Calculated T _m (°C)	Hybrid. efficiency (%)	Specificity	Target
U	ATGGATTAAAGTCCAT	17	62	0.06	+
U1	CAATGGATTAAAGTCCATCGC	22	69	0.05	+
α	TTTAAAGTCCATCGCC	16	68	0.3	+
α1	TTTAAAGTCCATCGCCTTAA	20	69	3	-
α2	TTTAAAGTCCATCGCCTTAACCA	23	74	8	-
D3	GCCTTAACCACTCGGCCA	18	83	100	-

FIGURE 2. Properties of oligonucleotide probes used for the detection of serine suppressor tRNA. Melting temperatures for the DNA/RNA hybrid duplexes were calculated at the total oligonucleotide concentration of 100 μ M in 1 M NaCl according to the nearest-neighbor method of Sugimoto et al. (1995). Hybridization efficiencies of oligonucleotides targeting the anticodon loop are reported as a percentage of the hybridization signal obtained using oligonucleotide D3 targeting the D loop. + and - indicate retention or loss of specificity for the tRNA^{su+}, respectively. Each oligonucleotide is depicted as a thick line along its tRNA^{su+} target region. Hybridization reactions on total RNA purified from 293 cells transfected with a plasmid carrying 16 copies of human serine suppressor tRNA gene were performed as described for oligonucleotide α .

Because TX is the only unfoldase whose hybridization does not appear to be influenced by the presence of modified nucleotides (data not shown), and more in vivo-made tRNA^{su+} is present on the gel, the ratio between the hybridization signals detected for the in vivo and in vitro tRNA^{su+} increased when compared with the unfoldase X1.

The unfolders D1 and D2, targeting the D arm and hybridizing with 3 nt of the D stem, were as active as unfoldase TX when tested on the in vitro-transcribed tRNA^{su+}. However, their efficacy did not improve proportionally on the in vivo-made tRNA^{su+} (with increases of 66- and 54-fold, respectively), as the highly modified D loop can reduce their hybridization efficiency (Fig. 1). According to this view, the activity of the shorter and less stable unfoldase D2 (Fig. 3) was more affected by this constraint.

When the hybridization efficiency of the labeled unfoldases X and TX was measured on the in vitro and in vivo-made tRNA^{su+}, we found that the hybridization of X was reduced by approximately fivefold by the presence of a m³C modification at position 47:3 of the extra arm (Fig. 1; data not shown). However, their difference in enhancing the hybridization of oligonucleotide α was 73-fold (Fig. 4A). Taken together, these results suggest that the most active unfoldases presumably displace the

strands of the D and anticodon stems and consequently promote an efficient heteroduplex formation.

The fact that single unfoldases were active in enhancing the hybridization of oligonucleotide α prompted us to examine their potential synergistic effects. As shown in Figure 4B, four different unfoldase combinations were tested. The combination of unfoldases targeting the acceptor/T Ψ C arm and the extra arm (T1 + X, T2 + X) that were poorly active when employed singularly (Fig. 4A) were capable of increasing the hybridization of oligonucleotide α on the in vivo-made tRNA^{su+} up to sevenfold (Fig. 4B). Both combinations of unfoldases do not hybridize with the anticodon stem, but together, they can potentially disrupt the interactions occurring between the D loop and the T Ψ C/extra arms responsible for the tRNA tertiary structure (G15:C48, G19:C56; Heckl et al., 1998; Fig. 1). Accordingly, as the activity of the unfoldase X1 increased just threefold by the presence of T1, it appears that the disruption of the tRNA tertiary structure can slightly enhance anticodon hybridization. As expected, the combination T1 + X1 that partially reconstructs the TX unfoldase activated oligonucleotide α hybridization with the same efficiency of TX.

When the shortest unfoldases D2 and X1 targeting the D and anticodon stems and theoretically able to disrupt

Unfolder	Sequence (5'→3')	Length	Calculated T _m (°C)	Target
T1	GGCGTAGTCGGCAGGATTCGAA	22	81
T2	GCAGGATTCGAACCTGC	17	75
X	GCGGGGAGACCCCA	14	84
X1	GGAGACCCCAATGGA	15	74
TX	GTCGGCAGGATTCGAACCTGCGCGGGGAGACCCCAATGGA	40	91
D1	TTAACCACTCGGCCACGACTAC	22	77
D2	TTAACCACTCGGCCAC	16	73

Acceptor arm	TΨC arm	Extra arm	Anticodon arm	D arm
ΔG= -14.3	ΔG= -6.5	ΔG= -4.2	ΔG= -3.1	ΔG= -2.4

FIGURE 3. Properties of oligonucleotides used as unfolders and thermodynamic analysis of different tRNA^{Ser} regions. Melting temperatures for the DNA/RNA hybrid duplexes were calculated as in Figure 2. Each unfolded is depicted as a thick line along its tRNA^{Ser} target region. ΔG of the five serine tRNA^{Ser} arms was calculated using the *mfold* program, version 3 (Mathews et al., 1999; Zuker et al., 1999).

the base pairing of these regions were employed, a 70- and 220-fold increases in oligonucleotide α hybridization were observed on in vitro-transcribed and in vivo-made tRNA^{Ser}, respectively. In this experiment, the ratio between hybridization signals decreases because of the negative effect of modified nucleotides on the hybridization of the unfolders and the labeled oligonucleotide α .

Finally, as clearly shown in Figure 4A,B (gel images, middle lanes, RNA purified from untransfected cells), the specificity of oligonucleotide α for the tRNA^{Ser} was not altered by the presence of any unfolded combination.

Our results demonstrate that tRNA anticodon hybridization can be greatly improved by the action of unfolded oligonucleotides that, through reshaping the structure of the tRNA, improve heteroduplex formation. A similar concept has been used in the past for facilitating RNA cleavage by RNase H and 2'-O-methyl oligonucleotides (Hayase et al., 1990) or for hammerhead ribozymes (Jankowsky & Schwenzer, 1996). However, this is the first time that this approach has been applied to the detection of specific tRNA isoacceptors.

When a pair of oligonucleotides hybridizing the regions flanking the target site of a ribozyme was em-

FIGURE 4. Unfolder oligonucleotides enhance the hybridization efficiency of an oligonucleotide probe complementary to the tRNA^{Asu+} anticodon loop. Gray bars and black bars correspond, respectively, to the *in vitro*-transcribed tRNA^{Asu+} or tRNA^{Asu+} made in mammalian cells. The gray thick lines along the tRNA^{Asu+} correspond to the 5'-end-labeled oligonucleotide α ; the black thick lines correspond to different cold unfolders employed in the pre/hybridization reactions. Two hybridization signals were detected by oligonucleotide α . The characterization of the slower migrating band that corresponds to the tRNA^{Asu+} precursor carrying the 3' trailer is reported in Buvoli et al. (2000). This signal was not included in the calculation of the hybridization efficiency of oligonucleotide α . Hybridization signals shown at the bottom of the figure correspond, from the left to the right, to the 88-nt-long *in vitro*-transcribed tRNA^{Asu+} (2 ng), 10 μ g of total RNA purified from untransfected cells, and 10 μ g of total RNA purified from cells transfected with a plasmid carrying 16 copies of human serine suppressor tRNA gene, containing ~28 ng of the 85-nt-long tRNA^{Asu+}. Hybridization signals were normalized to the level of 5.8S rRNA and quantified as described in Materials and methods. Hybridization efficiency is expressed as pixel counts. **A:** Hybridization efficiency of oligonucleotide α measured in the presence of single unfolders. **B:** Hybridization efficiency of oligonucleotide α measured in the presence of different combinations of unfolders.

played, a 115- or 4–12-fold increase in association rate between ribozyme and RNA was reported with long (~900 nt) or short (39–452 nt) substrates, respectively (Jankowsky & Schwenzner, 1996). Here we

show that the presence of 100-fold molar excess of cold unfolders over the tRNA^{Asu+} increases the hybridization signal of an oligonucleotide complementary to the anticodon loop up to 220-fold. Although

the anticodon region contains unpaired bases that should allow an efficient oligonucleotide nucleation, it appears that productive heteroduplex formation is sterically inhibited by the unique structural characteristics of the anticodon stem (Mir & Southern, 1999). Our data suggest that the anticodon also maintains an inhibitory arrangement when the tRNA tertiary interactions are disrupted by unfolders targeting the T Ψ C and the extra arms. Thus, the separation of the anticodon stem strands appears to be an efficient way to promote heteroduplex formation. When the tRNA secondary structures interfering with oligonucleotide α hybridization were completely eliminated, we observed the highest heteroduplex yield. Interestingly, in this assay, a cooperative effect between unfolders was observed. In fact, the hybridization signal detected in the presence of D2 and X1 was ~ 2.5 -fold higher than expected for a simple additive effect of these unfolders. This finding could reflect a more complex hybridization picture in which oligonucleotide α can play an active role in promoting the hybridization of one of the two unfolders with the formation of a more stable heteroduplex complex (for example: tRNA + α + D2 + X1 $\rightleftharpoons$ tRNA/D2/ α + X1 $\rightarrow$ tRNA/D2/ α /X1). This potential effect probably reduces the need for the use of long unfolders like TX.

Our results show that the presence of unfolders allows the use of short oligonucleotide probes directed against tRNA regions that were previously inaccessible for hybridization (Kumazawa et al., 1992; Mir & Southern, 1999) and suggest that the same approach could be successfully employed for the detection of other highly structured RNAs.

Our technique, introducing a great level of flexibility in designing tRNA probes that can discriminate between isoacceptors that differ by a single nucleotide, facilitates the study of recombinant tRNAs as well as the expression analysis of endogenous tRNAs.

MATERIALS AND METHODS

Plasmids

Plasmid ptRNA16mer/105, containing 16 copies of human serine suppressor tRNA gene, is described in Buvoli et al. (2000).

Cell transfection and RNA isolation

We grew 293 cells in Dulbecco's Modified Eagle Medium (Gibco BRL), supplemented with 10% fetal bovine serum (HyClone). At a confluency of 60–80%, cells were transfected with 6 μ g of plasmid according to the LIPOFECTAMINE Reagent (Life Technologies) procedure. After a 36-h incubation at 37 °C, total RNA was isolated with TRI REAGENT (Molecular Research Center), according to the manufacturer's protocol. RNA was resuspended in H₂O and stored at –70 °C.

RNA analysis

Ten micrograms of total RNA (containing ~ 28 ng of the 85-nt-long tRNA^{su+}) purified from cells transfected with a plasmid carrying 16 copies of human serine suppressor tRNA gene (Buvoli et al., 2000), 10 μ g of total RNA purified from untransfected cells, and 2 ng of the in vitro-transcribed tRNA^{su+} (88 nt long) were lyophilized and resuspended in 5 μ L of formamide, 0.03% bromophenol blue, and 0.03% xylene cyanole. Samples were denatured at 85 °C for 5 min, chilled quickly, and subsequently applied multiple times on an 8 M urea, 8% polyacrylamide gel run at 55 °C. The portion of the gel containing the RNA of interest was then electroblotted onto a Nytran SuperCharge nylon membrane (Schleicher & Schuell) in TBE buffer for 3 h at 500 mA, using the Hoefer TE 22 mini tank transphor unit. The membrane, UV crosslinked according to the manufacturer's instructions using the Stratalinker UV Crosslinker (Promega), was subsequently cut in several parts each containing the three RNA species described above. tRNA^{su+} was detected by Northern blot hybridization using the hot oligonucleotide α (Fig. 2) alone, or in combination with cold unfolders (Fig. 3). Oligonucleotide α was labeled at the specific activity of $> 8 \times 10^8$ dpm/ μ g with polynucleotide kinase and [γ^{32} P]-ATP (5,000 Ci mmol; NEN) according to Sambrook et al. (1989). Membranes were first prehybridized for 2 h at 42 °C in 2 $\times$ SSC and solution H (10 $\times$ Denhardt's solution, 0.1% SDS, 100 mg/mL denatured salmon and sperm DNA). Each unfolders was then added at a concentration of 24 nM, and prehybridization was continued for three more hours. The following hybridization reaction was carried out overnight at 27 °C in 6 $\times$ SSC, solution H, 20 nM of specific unfolders, and oligonucleotide α (3×10^6 cpm/mL). After hybridization, membranes were washed 4 $\times$ 15 min in 6 $\times$ SSC at room temperature. Hybridization signals were analyzed by a Storm 860 image analyzer (Molecular Dynamics) using the ImageQuant version 5.1 software. Hybridization signals were normalized to the level of 5.8S rRNA after membranes were stripped and reprobed with oligo R (5'-CGAAGTGTCTGATGATCAAT-3') complementary to nt 86–104 of the 5.8S rRNA.

In vitro transcription

In vitro transcription of human serine tRNA^{su+} was carried out according to Milligan and Uhlenbeck (1989) on a PCR fragment containing the T7 promoter. The in vitro-transcribed tRNA^{su+} is 88 nt long, contains the CCA sequence at its 3' end, and three extra nucleotides at the 5' end (GGG) derived from the consensus sequence of the T7 promoter (Brown et al., 1986).

ACKNOWLEDGMENTS

This work has been supported by a grant from the Muscular Dystrophy Association to M.B. and National Institutes of Health grant HL50560 to L.A.L. We thank Olke Uhlenbeck for many stimulating discussions and Mike Yarus for critical reading of the manuscript.

Received February 16, 1999; returned for revision March 25, 2000; revised manuscript received April 7, 2000

REFERENCES

- Achsel T, Gross HJ. 1993. Identity determinants of human tRNA^{ser}: Sequence elements necessary for serylation and maturation of a tRNA with a long extra arm. *EMBO J* 12:3333–3338.
- Brown JP, Klement JF, McAllister WT. 1986. Sequence of three promoters for the bacteriophage SP6 RNA polymerase. *Nucleic Acids Res* 14:3521–3526.
- Buvoli M, Buvoli A, Leinwand AL. 2000. Suppression of nonsense mutations in cell culture and mice by multimerized suppressor tRNA genes. *Mol Cell Biol* 20:3116–3124.
- Capone JP, Sharp PA, RajBhandary UL. 1985. Amber, ochre and opal suppressor tRNA genes derived from a human serine tRNA gene. *EMBO J* 4:213–221.
- Crothers DM, Cole PE, Hilbers CW, Shulman RG. 1974. The molecular mechanism of thermal unfolding of *Escherichia coli* formyl-methionine transfer RNA. *J Mol Biol* 87:63–88.
- Dirheimer G, Baranowski W, Keith G. 1995. Variations in tRNA modification, particularly of their queuine content in higher eukaryotes. Its relation to malignancy grading. *Biochimie* 77:99–103.
- Graeber MB, Muller U. 1998. Recent developments in the molecular genetics of mitochondrial disorders. *J Neurol Sci* 153:251–263.
- Hatlen L, Attardi G. 1971. Proportion of the HeLa cell genome complementary to transfer RNA and 5S RNA. *J Mol Biol* 56:535–553.
- Hayase Y, Inoue H, Ohtsuka E. 1990. Secondary structure in formyl-methionine tRNA influences the site-directed cleavage of ribonuclease H using chimeric 2'-O-methyl oligodeoxyribonucleotides. *Biochemistry* 29:8793–8797.
- Heckl M, Busch K, Gross HJ. 1998. Minimal tRNA^{ser} and tRNA^{sec} substrates for human seryl-tRNA synthetase: Contribution of tRNA domains to serylation and tertiary structure. *FEBS Lett* 427:315–319.
- Jankowsky E, Schwenzer B. 1996. Efficient improvement of hammerhead ribozyme mediated cleavage of long substrates by oligonucleotide facilitators. *Biochemistry* 35:15313–15321.
- Kumazawa Y, Yokogawa T, Tsurui H, Miura K, Watanabe K. 1992. Effect of the higher-order structure of tRNAs on the stability of hybrids with oligodeoxyribonucleotides: Separation of tRNA by an efficient solution hybridization. *Nucleic Acids Res* 20:2223–2232.
- Mathews DH, Sabina J, Zuker M, Turner DH. 1999. Expanded sequence dependence of thermodynamic parameters provides robust prediction of RNA secondary structure. *J Mol Biol* 288:911–940.
- Milligan JF, Uhlenbeck OC. 1989. Synthesis of small RNAs using T7 RNA polymerase. *Methods Enzymol* 180:51–62.
- Mir KU, Southern EM. 1999. Determining the influence of structure on hybridization using oligonucleotide arrays. *Nat Biotechnol* 17:788–792.
- Mukerjee H, Goldfeder A. 1976. Transfer RNA species in tumors of different growth rates. *Cancer Res* 36:3330–3338.
- Petyuk VA, Zenkova A, Giege R, Vlassov VV. 1999. Hybridization of antisense oligonucleotides with the 3' part of the tRNA^{Phe}. *FEBS Lett* 444:217–221.
- Saier MH Jr. 1995. Differential codon usage: A safeguard against inappropriate expression of specialized genes? *FEBS Lett* 362:1–4.
- Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*. 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Sprague KU. 1995. Transcription of eukaryotic tRNA genes. In: Söll D, RajBhandary UT, eds. *tRNA: Structure, biosynthesis, and function*. Washington, DC: American Society for Microbiology. pp 31–50.
- Söll D. 1993. Transfer RNA: An RNA for all seasons. In: Gesteland RF, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 157–183.
- Sprinzi M, Grueter F, Spelzhaus A, Gauss DH. 1980. Compilation of tRNA sequences. *Nucleic Acids Res* 8:r1–r22.
- Sugimoto N, Nakano S, Katoh M, Matsumura A, Nakamuta H, Ohmichi T, Yoneyama M, Sasaki M. 1995. Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. *Biochemistry* 34:11211–11216.
- Varshavsky A. 1996. The N-end rule: Functions, mysteries, uses. *Proc Natl Acad Sci USA* 93:12142–12149.
- White RJ. 1997. Regulation of RNA polymerase I and III by the retinoblastoma protein: A mechanism for growth control? *Trends Biochem Sci* 22:77–80.
- Zuker M, Mathews DH, Turner DH. 1999. Algorithms and thermodynamics for RNA secondary structure prediction: A practical guide. In: Barciszewski J, Clark BFC, eds. *RNA biochemistry and biotechnology*. NATO ASI Series. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp 11–43.