The EMBO Journal vol.13 no.13 pp.3166–3169, 1994

The exchange of the discriminator base A73 for G is alone sufficient to convert human tRNA^{Leu} into a serine-acceptor *in vitro*

Kristin Breitschopf and Hans J.Gross[1]

Institut für Biochemie, Bayerische Julius-Maximilians-Universität, Biozentrum, Am Hubland, D-97074 Würzburg, Germany

[1]Corresponding author

Communicated by H.J.Gross

Transfer RNA (tRNA) identity is maintained by the highly specific interaction of a few defined nucleotides or groups of nucleotides, called identity elements, with the cognate aminoacyl-tRNA synthetase, and by non-productive interactions with the other 19 aminoacyl-tRNA synthetases. Most tRNAs have a set of identity elements in at least two locations, commonly in the anticodon loop or in the acceptor stem, and at the discriminator base position 73. We have used T7 RNA polymerase transcribed tRNAs to demonstrate that the sole replacement of the discriminator base A73 of human tRNA^{Leu} with the tRNA^{Ser}-specific G generates a complete identity switch to serine acceptance. The reverse experiment, the exchange of G73 in human tRNA^{Ser} for the tRNA^{Leu}-specific A, causes a total loss of serine specificity without creating any leucine acceptance. These results suggest that the discriminator base A73 of human tRNA^{Leu} alone protects this tRNA against serylation by seryl-tRNA synthetase. This is the first report of a complete identity switch caused by an exchange of the discriminator base alone.

Key words: discriminator base/human tRNA^{Ser} and tRNA^{Leu}/tRNA identity

Introduction

Transfer RNA (tRNA) molecules have similar sizes and tertiary structures, mainly due to their specific role in ribosomal protein biosynthesis. Class I tRNAs contain a variable region of only four to five nucleotides, whereas tRNAs of class II, including tRNA^{Leu}, tRNA^{Ser}, selenocysteine-specific tRNA (tRNA^{Sec}) and prokaryotic tRNA^{Tyr}, contain at least 11 nucleotides which form an additional stem and loop, the long extra arm (Steinberg *et al.*, 1993). All tRNAs of one family and most tRNAs accepting chemically similar amino acids are characterized by an identical, phylogenetically well-conserved residue at position 73, the discriminator base (Crothers *et al.*, 1972). It has been shown that this position contributes to the identity of most tRNAs (Normanly and Abelson, 1989; Schulman, 1991; Giegé *et al.*, 1993; McClain, 1993a). tRNA identity has been studied most thoroughly in *Escherichia coli*, where recognition elements are mainly located in two tRNA domains: in (i) the discriminator base position 73 and (ii) the acceptor stem or the anticodon loop, and far less frequently in the variable pocket or in the extra arm (Normanly and Abelson, 1989; Schimmel, 1989; Schulman, 1991; Giegé *et al.*, 1993; McClain, 1993a,b; Saks *et al.*, 1994).

We have recently characterized the identity elements of human tRNA^{Ser} (Achsel and Gross, 1993; Wu and Gross, 1993): the discriminator base G73, the long extra arm which acts in an orientation-dependent, but not sequence-specific, manner, and minor recognition sequences. Surprisingly, the long extra arms of human tRNAs^{Ser} and tRNAs^{Leu} have the same number of unpaired nucleotides at the 5′ and 3′ sides of this structural domain, thus resulting in a similar orientation. Moreover, tRNA^{Leu} contains most of the minor identity elements of tRNA^{Ser} (Achsel and Gross, 1993) in the DHU arm and in the TΨC arm. We show here that the exchange of the discriminator base A73 for the tRNA^{Ser}-specific G73 is alone sufficient to convert human tRNA^{Leu} into a serine acceptor and to eliminate all leucine specificity.

Results

A human tRNA^{Leu} with the serine-specific discriminator base G is an efficient serine acceptor

Human tRNA^{Sec} (Figure 1A) and tRNA^{Ser} (Figure 1B) are serylated by the same seryl-tRNA synthetase (Wu and Gross, 1993). In contrast to the isoacceptors of other tRNA species, tRNA^{Sec} is strikingly different from tRNA^{Ser} in its primary, secondary and tertiary structure (Sturchler *et al.*, 1993; Figure 1A). Besides the discriminator base G73, the long extra arms of human tRNA^{Sec} and tRNA^{Ser} function as major identity elements for serylation in an orientation-dependent, but not sequence-specific, manner (Achsel and Gross, 1993; Wu and Gross, 1993). The human tRNA^{Leu} isoacceptors (Green *et al.*, 1990) also have a long extra arm in the same orientation as human tRNAs^{Ser} (Steinberg *et al.*, 1993). This suggests that the discriminator base A73 of tRNA^{Leu} may play a dominant role as an identity element.

A suitable approach to examine tRNA identity is the transcription of tRNA genes with T7 RNA polymerase. These transcripts are completely unmodified, but nevertheless they are generally good substrates for aminoacylation (Milligan and Uhlenbeck, 1989; Sampson and Uhlenbeck, 1988). Synthetic tRNA^{Ser} (Wu and Gross, 1993) and tRNA^{Leu} wild-type genes were transcribed by T7 RNA polymerase. Both run-off transcripts (Figure 1B and C) are efficiently aminoacylated with serine and leucine, respectively, but not vice versa. It should be noted that for an efficient aminoacylation, all T7 transcripts of wild-type tRNA^{Ser}, wild-type tRNA^{Leu} and their derivatives should be denatured and slowly renatured before addition to the aminoacylation assay (Sampson and Uhlenbeck, 1988). The synthetic wild-type tRNA genes were taken as substrates for an exchange of the discriminator bases

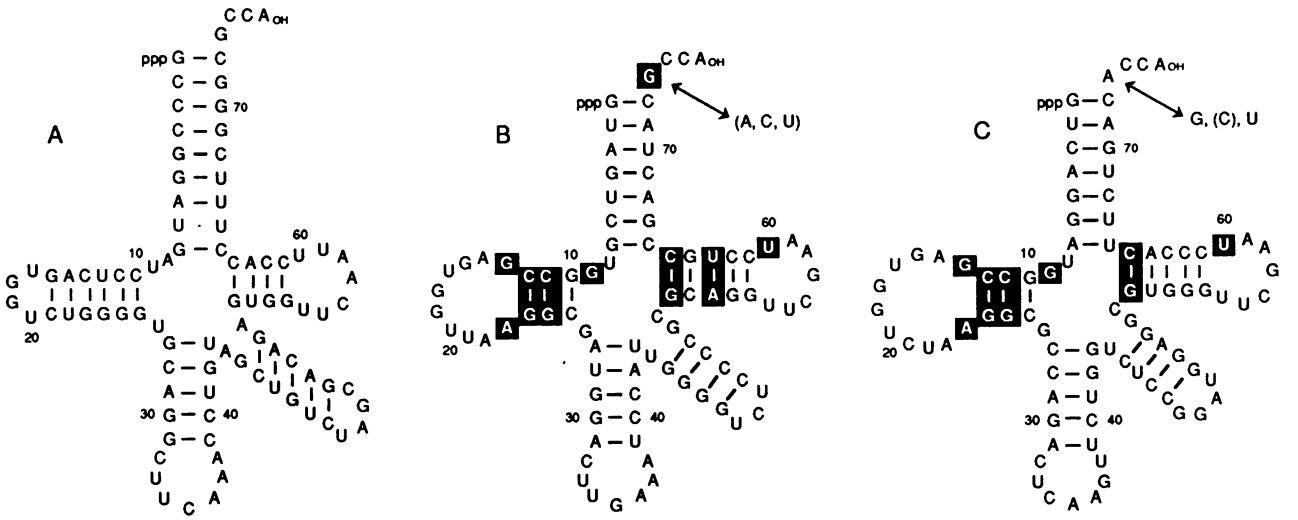

Fig. 1. Nucleotide sequence and secondary structure of unmodified human tRNA^{Sec} and of *in vitro* synthesized human tRNA^{Ser} and human tRNA^{Leu}. (**A**) tRNA^{Sec} [secondary structure and numbering according to Sturchler *et al.* (1993)]. (**B**) tRNA^{Ser} with UGA anticodon (Capone *et al.*, 1985). (**C**) tRNA^{Leu} with CAA anticodon (Green *et al.*, 1990). Identity elements of human tRNA^{Ser} (Achsel and Gross, 1993), except the long extra arm, are indicated by white letters on black background. They are marked in the same way as they also occur in human tRNA^{Leu}. Arrows indicate the substitutions made in this study: tRNA^{Ser} A73, C73 and U73, and tRNA^{Leu} G73, C73 and U73. The tRNA mutants with discriminator bases (position 73) in brackets are inactive.

by *in vitro* mutagenesis. Base G73 of tRNA^{Ser} was replaced by A, C or U (Figure 1B), whereas residue A73 of tRNA^{Leu} was exchanged for G, C or U (Figure 1C).

tRNAs^{Ser} A73, C73 and U73, and tRNA^{Leu} C73 are neither charged with serine nor with leucine upon aminoacylation with HeLa S100 extract. tRNA^{Leu} U73 is very inefficiently charged with leucine but not with serine (not shown). Surprisingly, tRNA^{Leu} G73 is an efficient serine acceptor, whereas aminoacylation with leucine is completely abolished (Figure 2). A V_{max}/K_m of 0.11 for serylation as compared with the wild-type tRNA^{Ser} transcript (V_{max}/K_m defined as 1.0) was determined (Table I). The kinetic data (Figure 2) show no difference between the relative V_{max} of tRNA^{Ser} and tRNA^{Leu} G73, indicating that the initial interaction of tRNA^{Leu} G73 with seryl-tRNA synthetase is complex, and that the rate of product formation is equal to that of wild-type tRNA^{Ser} whenever a productive interaction occurs.

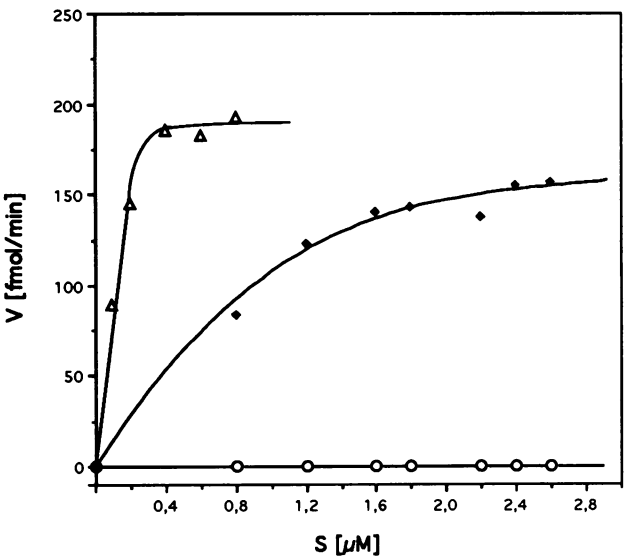

Fig. 2. Kinetics of aminoacylation: Michaelis–Menten plot of human tRNA^{Ser} (△) and tRNA^{Leu} G73 (◆) serylation and leucine acceptance of tRNA^{Leu} G73 or tRNA^{Leu} with a short extra arm of five nucleotides (○) in HeLa cytoplasmic S100 extract. Five and six tRNA concentrations, between 0.1 and 0.8 μM for wild-type tRNA^{Ser} and 0.8 and 2.6 μM for tRNA^{Leu} G73, respectively, were used for kinetic studies shown above.

Discussion

There are only a few reports on altered tRNA aminoacylation caused by single nucleotide changes. They mainly refer to *E.coli* tRNA^{Trp} (Yaniv *et al.*, 1974) and *E.coli* tRNA^{Asn} (Li *et al.*, 1993). A single base change in the centre of the anticodon (position 35) of tRNA^{Trp} alters not only the translational specificity of that tRNA from UGG to UAG, but also its acceptor specificity from tryptophan to glutamine *in vivo* (Yaniv *et al.*, 1974). Substitution of nucleotide G34 with C34 converts the tRNA^{Asn} into a lysine acceptor *in vivo*, whereas substitution of the discriminator base G73 with A73 only reduces asparagine-specific aminoacylation (Li *et al.*, 1993).

The significance of the discriminator base G73 for *E.coli* tRNA^{Ser} is not clearly determined. On the one hand it has no crucial effect on serylation *in vitro* (Shimizu *et al.*, 1992), on the other hand eight base changes in the acceptor stem, including the discriminator base, and in the DHU stem are necessary for an identity switch from a suppressor tRNA^{Leu} to serine specificity *in vivo* (Normanly *et al.*, 1992). The nucleotides in the acceptor

Table I. Serylation kinetics of unmodified tRNA^{Ser} and tRNA^{Leu} G73

tRNA	Relative V_{max} (pmol/min)	Approx. K_m (μM)	V_{max}/K_m
tRNA^{Ser}	0.25	0.17	1.00
tRNA^{Leu} G73	0.26	1.75 ± 0.10	0.11

Each parameter was derived from a Lineweaver–Burke plot. V_{max}/K_m = 1.0 for tRNA^{Ser} is used as standard.

stem of *E.coli* $tRNA^{Ser}$ are absolutely necessary for serylation. The exchange of the first and of the third base pair strongly reduces the productive interaction with seryl-tRNA synthetase and increases that with glutaminyl-tRNA synthetase (Rogers and Söll, 1988). As shown by recent work on human $tRNA^{Ser}$ identity (Achsel and Gross, 1993) and by our constructs ($tRNA^{Leu}$ G73, $tRNA^{Ser}$ A73, C73, U73; Figure 1B and C), residue G73 is absolutely essential for human $tRNA^{Ser}$ and cannot be replaced by any other of the three bases without complete loss of serine acceptor activity. The discriminator base A73 of *E.coli* $tRNA^{Leu}$ serves as a critical recognition site for leucyl-tRNA synthetase in a similar, but less decisive, way. Replacement by any other base leads only to reduced aminoacylation with leucine (Asahara *et al.*, 1993).

A synopsis of the nucleotide sequences of human $tRNA^{Ser}$ and $tRNA^{Leu}$ isoacceptors (Figure 1B and C) reveals that the major and minor identity elements of human $tRNA^{Ser}$ (Achsel and Gross, 1993), except G73, are also present in the sequence of $tRNA^{Leu}$. In both $tRNA^{Ser}$ and $tRNA^{Leu}$ one unpaired nucleotide at the 5′ and two nucleotides at the 3′ site of the long extra arm define its orientation. Some of the minor identity elements of human $tRNA^{Ser}$ (Achsel and Gross, 1993), G49:C65, G9 and U60 also appear in $tRNA^{Leu}$. Different numbers of unpaired nucleotides occur at the base of the long extra arm in *E.coli* class II tRNAs (Steinberg *et al.*, 1993). The orientation of this structural domain and consequently the tertiary structure of these tRNAs could, therefore, be quite different among each other. For instance, a complete identity switch from *E.coli* $tRNA^{Tyr}$ to serine acceptance was only achieved by an exchange of nucleotides at positions 9 and 73 and by an alteration of the orientation of the long extra arm (Himeno *et al.*, 1990). However, the influence of the long extra arm in $tRNA^{Leu}$ seems to be ambiguous. An *E.coli* amber suppressor $tRNA^{Leu}$ with a short extra arm of five nucleotides is aminoacylated exclusively and efficiently with leucine *in vivo* (Normanly *et al.*, 1992), quite in contrast to our results, whereas aminoacylation of the human $tRNA^{Leu}$ with a short extra arm of five nucleotides is completely abolished (Figure 2).

The localization of nucleotides G18 and G19 in the DHU loop is different in $tRNA^{Leu}$ and $tRNA^{Ser}$. Unlike human serine-specific isoacceptors, $tRNAs^{Leu}$ contain one additional nucleotide in the DHU loop and one in the loop of the long extra arm (Figure 1C). These minor differences should result in subtle structural changes of the tRNA molecule. Specifically, the structure of the variable pocket (McClain and Foss, 1988), consisting of bases at positions 16, 17, 20A, 59 and 60, could be affected. Seryl-tRNA synthetase seems to be unaffected by these minute structural changes. This notion is supported by the observation that both prokaryotic and eukaryotic $tRNAs^{Sec}$ are serylated by their homologous synthetases (Mizutani *et al.*, 1984; Leinfelder *et al.*, 1988; Wu and Gross, 1993). These tRNAs adopt an atypical secondary and tertiary structure (Sturchler *et al.*, 1993) which is different from all types of cytoplasmic tRNAs.

In summary, the discriminator base A73 of human $tRNA^{Leu}$ acts as a strongly negative identity element for seryl-tRNA synthetase. Its replacement by the serine-specific G73 alone is sufficient for an identity switch to serine acceptance and for the complete loss of leucine identity. The yet unknown structural demands of leucyl-tRNA synthetase seem to be quite different from those of seryl-tRNA synthetase, i.e. other mechanisms must exist for the discrimination between $tRNA^{Ser}$ and $tRNA^{Leu}$ by leucyl-tRNA synthetase. Our results demonstrate an unusually low requirement of seryl-tRNA synthetase for identity elements, as revealed by the efficient serylation of the three very different substrates $tRNA^{Sec}$, $tRNA^{Ser}$ and $tRNA^{Leu}$ G73 (Figure 1). This indicates that the human enzyme is distinguished from all other aminoacyl-tRNA synthetases by an exceptional flexibility in tRNA substrate recognition and interaction.

Materials and methods

Enzymes and reagents

T7 RNA polymerase was prepared from an overproducing strain kindly provided by Dr W.Studier according to established procedures (Zawadzki and Gross, 1991). HeLa cytoplasmic S100 extract was prepared as described by Dignam *et al.* (1983). All other enzymes were obtained from commercial suppliers. [3-^{3}H]serine (1.07 TBq/mmol) and [α-^{32}P]dATP were purchased from Amersham-Buchler, Braunschweig, Germany.

Bacterial strains and plasmids

Escherichia coli JM109 was used as a host for the propagation of plasmid pUC19 and its derivatives.

Construction of tDNA clones

The templates coding for $tRNA^{Ser}$ with UGA anticodon (Wu and Gross, 1993) and $tRNA^{Leu}$ with CAA anticodon, including the T7 promoter and the *Bst*NI recognition site, were assembled from synthetic oligonucleotides and cloned into pUC19. All subclones derived from these genes were constructed using appropriate synthetic oligodeoxynucleotides as polymerase chain reaction primers and were cloned into pUC19. The sequences of all constructs were confirmed by dideoxy sequencing (Sanger *et al.*, 1977).

Preparation of tDNA transcripts

Transcription of the *Bst*NI-linearized tDNA templates with T7 RNA polymerase yielded unmodified tRNAs with the correct 5′ and 3′ termini which were used for aminoacylation studies. The conditions for transcription with T7 RNA polymerase are described by Achsel and Gross (1993).

In vitro aminoacylation

All tRNAs (5 μM) were heated for 4 min to 68°C in 5 mM $MgCl_2$ and allowed to cool slowly to room temperature. Aminoacylation was performed at 37°C in 36 μl of a reaction mixture containing 20 mM imidazole–HCl (pH 7.5), 150 mM KCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5 mM CTP, 0.5 mM DTT, 3.3% (v/v) HeLa cytoplasmic S100 extract (8.1 mg protein/ml) and 5 μM serine, which included 0.7 μM [3-^{3}H]serine (1.07 TBq/mmol). tRNA concentrations ranged from 0.1 to 2.6 μM. Aliquots (6 μl) of the reaction mixture were transferred onto pieces of Whatman 3MM paper which were submitted to 10% trichloroacetic acid. [^{3}H]Amino acid not bound to tRNA was removed by two washes in 5% trichloroacetic acid and two washes in ethanol. Radiolabelled aminoacyl-tRNA was then measured by liquid scintillation counting. K_m and V_{max} values were obtained from a Lineweaver–Burke analysis of the initial rates by using five and six different tRNA concentrations, respectively.

Acknowledgements

We thank Prof. H.Beier and U.Weber for critically reading the manuscript and Drs T.Achsel and X.-Q.Wu for clones. This work was supported by Deutsche Forschungsgemeinschaft (SFB 165) and Fonds der Chemischen Industrie.

References

Achsel,T. and Gross,H.J. (1993) *EMBO J.*, **12**, 3333–3338.
Asahara,H., Tamura,K., Nameki,N., Himeno,H., Hasegawa,T. and

Shimizu,M. (1993) *J. Mol. Biol.*, **231**, 219–229.
Capone,J.P., Sharp,P.A. and RajBhandary,U.L. (1985) *EMBO J.*, **4**, 213–221.
Crothers,D.M., Seno,T. and Söll,D.G. (1972) *Proc. Natl Acad. Sci. USA*, **69**, 3063–3067.
Dignam,J.D., Lebovitz,R.M. and Roeder,R. (1983) *Nucleic Acids Res.*, **11**, 1475–1489.
Giegé,R., Puglisi,J.D. and Florentz,C. (1993) *Prog. Nucleic Acid Res. Mol. Biol.*, **45**, 129–206.
Green,J.C., Sohel,I. and Vold,B.S. (1990) *J. Biol. Chem.*, **265**, 12139–12142.
Himeno,H., Hasegawa,T., Ueda,T., Watanabe,K. and Shimizu,M. (1990) *Nucleic Acids Res.*, **18**, 6815–6819.
Leinfelder,W., Zehelein,E., Mandrand-Berthelot,M.-A. and Böck,A. (1988) *Nature*, **331**, 723–725.
Li,S., Pelka,H. and Schulman,L.H. (1993) *J. Biol. Chem.*, **268**, 18335–18339.
McClain,W.H. (1993a) *J. Mol. Biol.*, **234**, 257–280.
McClain,W.H. (1993b) *FASEB J.*, **7**, 72–77.
McClain,W.H. and Foss,K. (1988) *Science*, **241**, 1804–1807.
Milligan,J.F. and Uhlenbeck,O.C. (1989) *Methods Enzymol.*, **180**, 51–62.
Mizutani,T., Narihara,T. and Hashimoto,A. (1984) *Eur. J. Biochem.*, **143**, 9–13.
Normanly,J. and Abelson,J. (1989) *Annu. Rev. Biochem.*, **58**, 1029–1049.
Normanly,J., Ollick,T. and Abelson,J. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 5680–5684.
Rogers,M.J. and Söll,D. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 6627–6631.
Saks,M.E., Sampson,J.R. and Abelson,J.N. (1994) *Science*, **263**, 191–197.
Sampson,J.R. and Uhlenbeck,O.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 1033–1037.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl Acad. Sci. USA*, **12**, 5335–5339.
Schimmel,P. (1989) *Biochemistry*, **28**, 2747–2759.
Schulman,L.H. (1991) *Prog. Nucleic Acid Res. Mol. Biol.*, **41**, 23–87.
Shimizu,M., Asahara,H., Tamura,K., Hasegawa,T. and Himeno,H. (1992) *J. Mol. Evol.*, **35**, 436–443.
Steinberg,S., Misch,A. and Sprinzl,M. (1993) *Nucleic Acids Res.*, **21**, 3011–3015.
Sturchler,C., Westhof,E., Carbon,P. and Krol,A.(1993) *Nucleic Acids Res.*, **21**, 1073–1079.
Wu,X.-Q. and Gross,H.J. (1993) *Nucleic Acids Res.*, **21**, 5589–5594.
Yaniv,M., Folk,W.R., Berg,P. and Soll,L. (1974) *J. Mol. Biol.*, **86**, 245–260.
Zawadzki,V. and Gross,H.J. (1991) *Nucleic Acids Res.*, **19**, 1948.

Received on March 28, 1994; revised on May 3, 1994