291 to 300, 329 to 335, and 403 to 405, as well as residues 379 to 381, which actually form a β strand. Another conserved segment is 58 to 65, which is one of the segments of the N domain that is part of the active site. However, another of the conserved segments, residues 228 to 236, is not near the active site. It is at the back of the barrel, in contact with the COOH-terminus of the S subunit, which is also a conserved segment. The conservation of this region in L_8S_8 might be thought to reflect stringent demands of bonding of the two subunits. However, the conservation of 228 to 236 extends to *R. rubrum*, which lacks the S subunit. Also, among the more conserved regions are the contacts between domains B1 and N5, including residues 175 to 180, 209 to 213, 291 to 297, and 301 to 303. In general, conservation in the L subunit appears stronger for loops that surround the active site than for interdomain contacts, and somewhat stronger for interdomain contacts than for structural elements such as β strands or helices, although these classes of residues are not always distinct. In contrast, in the S subunit, conservation tends to be somewhat stronger in the β strands and helices than for the interdomain residues.

The pattern of intersubunit contacts is complicated. Each L subunit contacts four other L and three S subunits (L1 in Fig. 2C touches L3, L5, L7, L8, S1, S3, and S8); and each S subunit touches two other S and three L subunits (S1 contacts S7, S8, L1, L3, and L7). From the accessible atomic areas of these contacts, it is possible to estimate the hydrophobic energies of domain interaction (*26*). We find that the strongest hydrophobic interaction between domains is between the barrel of one subunit and the N domain that forms part of the same active site. An example is the B1-N5 interaction in Fig. 3C. These interaction energies and other aspects of RuBisCO structure will be discussed elsewhere.

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27. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K. Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Aminoacylation of Synthetic DNAs Corresponding to *Escherichia coli* Phenylalanine and Lysine tRNAs

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Synthetic DNA oligomers (tDNAs) corresponding to *Escherichia coli* $tRNA^{Phe}$ or $tRNA^{Lys}$ have been synthesized with either deoxythymidine (dT) or deoxyuridine (dU) substituted in the positions occupied by ribouridine or its derivatives. The tDNAs inhibited the aminoacylation of their respective tRNAs with their cognate amino acids, but not the aminoacylation of $tRNA^{Leu}$ with Leu. In the presence of aminoacyl-tRNA synthetase, species of both a $tDNA^{Phe}$ synthesized with a 3′ terminal riboadenosine and a $tDNA^{Lys}$ containing only deoxynucleotides could be aminoacylated with the appropriate amino acids, although the Michaelis constant K_m and observed maximal rate V_{max} values for aminoacylation were increased by three- to fourfold and decreased by two- to threefold, respectively. The aminoacylation of synthetic tDNAs demonstrates that the ribose backbone of a tRNA is not absolutely required for tRNA aminoacylation.

TRANSFER RNAS PLAY A PIVOTAL role in protein synthesis through their adaptor function and also serve as modulators of various cellular biosynthetic pathways (*1*). Because tRNAs are the most highly posttranscriptionally modified nucleic acid polymers (*2*), our laboratory has attempted to gain insight into the structural features important for tRNA function by determining how various modified nucleotides affect tRNA function (*3*). Recent advances in automated DNA synthesis (*4*) have increased our understanding of both the physical structure and the biological function of nucleic acids (*5*) as well as the mechanism of their interactions with themselves, with other nucleic acids, and with various cellular proteins (*6*). In an attempt to determine if the ribose backbone of tRNA is necessary for its biological functions, as well as to generate substrates that might be active with tRNA posttranscriptional modifying enzymes and to gain insight into the secondary and tertiary structure of tRNA genes, we synthesized DNA polymers that corresponded to the sense strand of the *Escherichia coli* $tRNA^{Phe}$ gene (*7, 8*) and the $tRNA^{Lys}$ gene (*9, 10*) to produce the corresponding full-length $tDNA^{Phe}$ and $tDNA^{Lys}$.

Initially we synthesized two 76-nucleotide DNA oligomers, corresponding to the full-length $tDNA^{Phe}$ with either dT or dU in the positions occupied by ribouridine or its derivatives in *E. coli* $tRNA^{Phe}$ (*7*). Since earlier studies demonstrated that the 3′ terminus of *E. coli* $tRNA^{Phe}$ requires the 2′ hydroxyl for aminoacylation (*11, 12*), it was not surprising that both of these tDNAs

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were not aminoacylated by *E. coli* Phe-tRNA synthetase (*13, 14*), although the dU-containing tDNA^{Phe} did inhibit the aminoacylation of tRNA^{Phe} with its cognate synthetase (*14*).

To further explore these initial observations, we synthesized two "*E. coli*" tDNA^{Phe} species that contained 75 deoxynucleotides (Fig. 1A), with either dU [denoted here as (dU) tDNA^{Phe}] or dT [(dT) tDNA^{Phe}] in the tDNA as described above, but with a riboadenosine as their 3′ terminal nucleotide. As shown in Fig. 2A, (dT) tDNA^{Phe} could be enriched approximately tenfold by fractionation on an RPC-5 column (*13, 15*) to yield a preparation with an average amino acid acceptor activity of 250 pmol per unit of absorbance at 260 nm (pmol/A_{260}) determined under optimal tDNA aminoacylation conditions (Table 1). Similar results were obtained for the (dU) tDNA^{Phe} species (*14*). The *p*H-dependent and buffer-dependent aminoacylation of the purified tDNA^{Phe} is shown in Fig. 3A. Attempts to obtain a tDNA preparation with greater than 15% aminoacylation activity (either by further column chromatography or by varying the aminoacylation incubation time, incubation temperature, enzyme concentration, or tDNA or Mg^{2+} concentration) were unsuccessful (*14*). We suspected that this low tDNA aminoacylation activity was partially due to the presence of a population of chemically modified, full-length tDNA species that was inactive in the aminoacylation reaction. When large oligonucleotides are synthesized and deblocked with the conditions and reagents usually used for automated DNA synthesis, various chemical modifications of the deoxynucleotides in the synthesized oligomer may occur (*16*). Even so, a significant portion of the full-length, chemically synthesized, and chromatographically purified tDNAs used in these present studies was active in the aminoacylation reaction under the optimal conditions (Table 1). Although dimethyl sulfoxide (DMSO) and spermidine are not absolutely required for complete aminoacylation of the tDNA^{Phe}, their inclusion in the aminoacylation reaction mixture increased the aminoacylation rate to the maximal level of approximately 15%. Prior treatment of either (dU) or (dT) tDNA^{Phe} with DNase I destroyed its aminoacylation ability, whereas a similar treatment with RNase T1 had little or no effect.

To further characterize the in vitro activity of the synthesized tDNAs, the purified (dT) tDNA^{Phe} was tested for its ability to inhibit the aminoacylation of tRNA^{Phe} and tRNA^{Leu}; (dT) tDNA^{Phe} inhibited the aminoacylation of tRNA^{Phe} by its cognate aminoacyl-tRNA synthetase, but did not inhibit the aminoacylation of tRNA^{Leu} (Fig. 4A). Similar results were obtained with (dU) tDNA^{Phe} (*14*). By assuming that competitive inhibition occurred, a Dixon

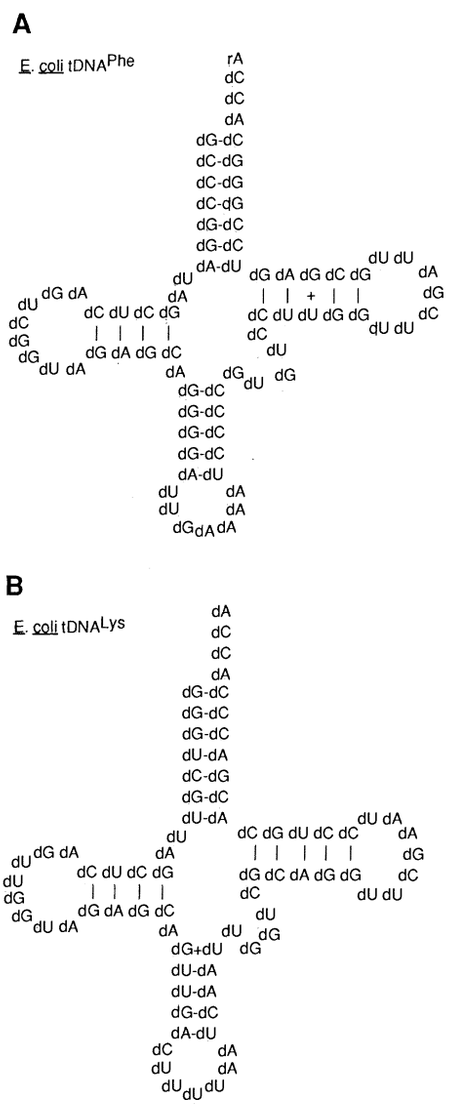

Fig. 1. The cloverleaf representation of the chemically synthesized oligodeoxynucleotides corresponding to (**A**) the full-length tDNA^{Phe} containing a 3′ terminal riboadenosine and (**B**) the tDNA^{Lys} containing entirely deoxyribonucleotides, with a deoxyuridine in the positions occupied by a ribouridine or its derivatives in *E. coli* tRNA^{Phe} (7) and *E. coli* tRNA^{Lys} (*9*), respectively.

Table 1. Effect of varying reaction conditions on the aminoacylation of tDNA^{Phe}, tRNA^{Phe}, tDNA^{Lys}, and tRNA^{Lys} by partially purified *E. coli* aminoacyl-tRNA synthetases. The complete aminoacylation reaction mixture for the tDNAs contained 0.01*M* cacodylate-KOH buffer, *p*H 5.5, 0.01*M* $MgCl_2$, 20% DMSO, 0.002*M* spermidine-HCl, 2.5 m*M* adenosine triphosphate (ATP), 0.01 m*M* ^{14}C-labeled amino acid (50 μCi/μmol), 0.1 mg of RPC-5–purified tDNA, and a saturating amount of aminoacyl-tRNA synthetase (*13*) in a total volume of 0.25 ml. The complete aminoacylation reaction mixture for the tRNAs contained 0.01*M* tris-HCl buffer, *p*H 7.6, 0.01*M* $MgCl_2$, 2.5m*M* ATP, 0.01 m*M* ^{14}O-labeled amino acid (50 μCi/μmole), 0.1 mg of DEAE-cellulose–purified total *E. coli* tRNA (*13*) isolated from HB101 cells that overproduced the tRNA^{Phe} from a plasmid containing the tRNA^{Phe} gene (*8, 13*), and a saturating amount of aminoacyl-tRNA synthetase (*13*) in a total volume of 0.25 ml. All of the reactions were incubated at 37°C for 30 min and terminated by adding 1.0 ml of ice-cold 10% trichloroacetic acid. The precipitates were collected by filtration onto Whatman GF/C glass-fiber disks and dried at 100°C for 10 min. The radioactivity retained on the disks was measured as previously described (*13, 28*). The results shown are the average of duplicate experiments, which deviated from the mean by less than 5%, and are corrected for endogenous tRNA acceptor activity present in the aminoacyl-tRNA synthetase preparation (*13*) and for nonspecific binding of radioactive amino acid to the filter by subtracting the results from parallel experiments incubated in the absence of added tDNA or tRNA.

Reaction mixture	Relative amino acid acceptor activity					
	tDNA^{Phe}		tRNA^{Phe}	tDNA^{Lys}		tRNA^{Lys}
	(dT)	(dU)		(dT)	(dU)	
Optimal reaction conditions*	100	100	100	100	100	100
Minus $MgCl_2$	5	4	5	6	4	5
Minus DMSO	56	53		57	53	
Plus DMSO			119			138
Minus spermidine-HCl	65	62		61	65	
Plus spermidine-HCl			116			114
Minus cacodylate-KOH, *p*H 5.5, plus tris-HCl, *p*H 7.6	14	17	100	14	15	100
Minus tris-HCl, *p*H 7.6, plus cacodylate-KOH, *p*H 5.5	100	100	7	100	100	7
tDNA pretreated with RNase T1*	96	93	2	93	91	4
tDNA pretreated with DNase I†	2	4	98	3	5	97

*In the RNase T1 pretreatment experiments, the nucleic acids were incubated in the presence of 50 U of RNAse T1 in 120 μl 10 m*M* tris-HCl, *p*H 7.6, for 5 min. The reaction was terminated by incubation at 100°C for 10 min. The RNase T1–treated nucleic acids were assayed for amino acid acceptor activity in the appropriate mixture described above. †In the DNase I pretreatment experiments, the nucleic acids were incubated in the presence of 3 μg of DNase 1 in 120 μl 10 m*M* tris-HCl, *p*H 7.6, for 5 min. The reaction was terminated by incubation at 100°C for 10 min. The DNase I–treated nucleic acids were assayed for amino acid acceptor activity in the appropriate mixture described above.

plot of the data (Fig. 4A) indicated that the apparent inhibition constant K_i is $\sim 0.3 \times 10^{-7} M$. Taken together, these results imply that the structural parameters necessary for the accuracy of the aminoacylation reaction are maintained in the tDNA, although the $tDNA^{Phe}$ has a deoxyribose backbone.

After the reaction conditions had been optimized and the initial inhibition studies were confirmed with the purified $tDNA^{Phe}$, the kinetics of tDNA aminoacylation were investigated. Kinetic analysis of the aminoacylation of the $tDNA^{Phe}$, at *p*H 5.5 with 10 m*M* Mg^{2+} in the presence of 20% DMSO and 2% spermidine to enhance the reaction, showed that the observed maximal rate V_{max} was one-third that obtained with $tRNA^{Phe}$ at *p*H 7.6 and 15 m*M* Mg^{2+} with similar DMSO or spermidine concentrations, and that the observed Michaelis constant K_m was threefold greater than that for $tRNA^{Phe}$ (Fig. 5A and Table 2). Additional analysis of the aminoacylation kinetics showed that, in the absence of DMSO and spermidine, the $tDNA^{Phe}$ at *p*H 5.5 had an observed V_{max} that was only one-half that of the observed V_{max} of $tRNA^{Phe}$ at *p*H 7.6, whereas the observed K_m again was threefold higher. These results in the presence of the organic solvent and polyamine are consistent with earlier studies of tRNA aminoacylation kinetics (*17*), which indicated that DMSO alters the aminoacyl-tRNA synthetase solution structure and thereby reduces synthetase specificity, whereas the polyamine specifically interacts with the tRNA and thus slightly alters the synthetase structure. The dU and dT forms of $tDNA^{Phe}$ had similar observed K_m values, whereas the observed V_{max} for (dU) $tDNA^{Phe}$ was higher than that for (dT) $tDNA^{Phe}$ but still less than the V_{max} obtained for the $tRNA^{Phe}$.

In a second series of experiments, we synthesized $tDNA^{Lys}$ containing either dU (Fig. 1B) or dT for ribouridine or its derivatives in *E. coli* $tRNA^{Lys}$ (*9*). Since $tRNA^{Lys}$ does not require the 2′ hydroxyl group on the 3′ terminal adenosine for its aminoacylation (*12*), both tDNAs were composed entirely of deoxynucleotides, including a 3′ terminal deoxyadenosine. Fractionation of (dT) $tDNA^{Lys}$ on RPC-5 (Fig. 2B) yielded a preparation that had an average Lys acceptor activity of 200 pmol/A_{260} unit under optimal conditions (Table 2). A similar tenfold purification on RPC-5 also was obtained for (dU) $tDNA^{Lys}$ (*14*). Thus both $tDNA^{Lys}$s can be aminoacylated by a partially purified preparation of aminoacyl-tRNA synthetase. The $tDNA^{Lys}$ had an optimum *p*H of 5.5 similar to that observed for the $tDNA^{Phe}$ (Fig. 3B and Table 1). The (dT) $tDNA^{Lys}$ inhibited the aminoacylation of $tRNA^{Lys}$ by its cognate aminoacyl-tRNA synthetase, but did not inhibit the aminoacylation of $tRNA^{Leu}$ (Fig. 4B). Similar results were obtained with (dU) $tDNA^{Lys}$ (*14*). A Dixon plot of the data (Fig. 4B) showed that the apparent K_i is $\sim 0.3 \times 10^{-7} M$. The results of a compara-

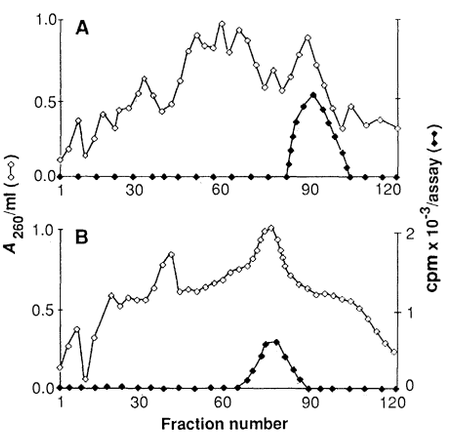

Fig. 2. Fractionation of totally synthetic (**A**) $tDNA^{Phe}$ and (**B**) $tDNA^{Lys}$. The $tDNA^{Phe}$ or $tDNA^{Lys}$ were synthesized (on an Applied Biosystems Automated DNA synthesizer, model 380A) with either a 5′ DMT-riboA^{bz}-3′-CPG or a 5′ DMT-deoxyriboA^{bz}-3′-CPG minicolumn, respectively, and deoxynucleotide cyanoethyl phosphoramidites (*4*). Approximately 8 mg of $tDNA^{Phe}$ or $tDNA^{Lys}$ were dissolved in 2 ml of TM buffer (0.01*M* tris-HCl, *p*H 7.6, and 0.01*M* $MgCl_2$) containing 0.3*M* NaCl and applied to a 0.8 cm by 50 cm (40 ml) RPC-5 column built and equilibrated in TM buffer containing 0.3*M* NaCl as previously described (*13*). The sample was eluted with a concave gradient of 0.3*M* NaCl (200 ml) to 1.2*M* NaCl (125 ml) in TM buffer at a flow rate of 2 ml per minute per fraction. The A_{260} of each fraction was measured and every fifth fraction was ethanol precipitated, vacuum dried, and redissolved in 0.2 ml of water prior to determining its amino acid acceptor activity (*13, 28*). A 0.025-ml aliquot of each redissolved fraction was assayed in triplicate for Phe or Lys acceptor activity (*13, 28*) (Table 1). The average values for the aminoacylation assays are plotted and the variation in the data was less than 5% overall.

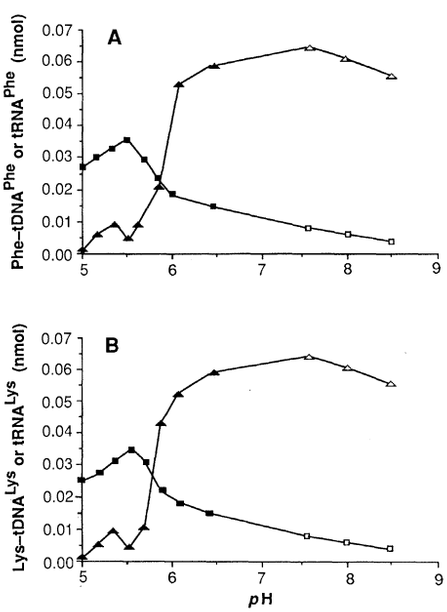

Fig. 3. The *p*H-dependent aminoacylation of purified (**A**) $tDNA^{Phe}$ and $tRNA^{Phe}$ and (**B**) $tDNA^{Lys}$ and $tRNA^{Lys}$. The values plotted are the averages of quadruplicate aminoacylation reactions, which varied by less than 5%, incubated with 0.2 A_{260} units of tDNA or tRNA in a 0.25-ml reaction at 37°C for 5 min under optimal conditions (Table 1) in the presence of either 0.01*M* cacodylate buffer titrated with 1.0*M* KOH or 0.01*M* tris buffer titrated with 1*M* HCl to the *p*H indicated. After incubation, the reactions were performed as described in Table 1, and the results from parallel reactions in the absence of tDNA or tRNA were subtracted prior to plotting the respective data points of the *p*H indicated: tDNA incubated in cacodylate-KOH (■—■) or in tris-HCl (□—□); and tRNA incubated in cacodylate-KOH (▲—▲) or in tris-HCl (△—△).

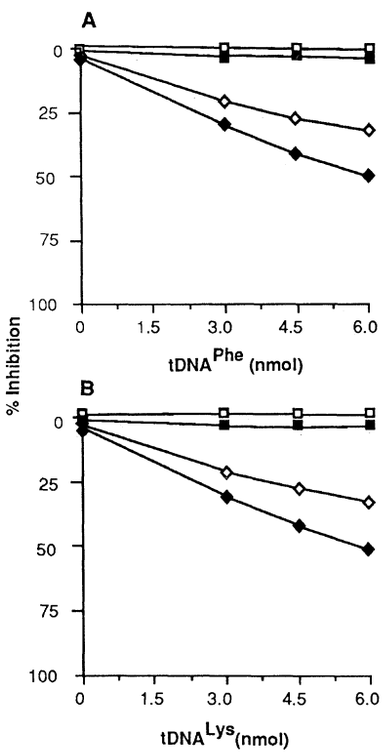

Fig. 4. The effect of increasing concentrations of (**A**) $tDNA^{Phe}$ on the amino acid acceptor activity of a partially purified preparation of $tRNA^{Phe}$ and $tRNA^{Leu}$; and (**B**) $tDNA^{Lys}$ on the amino acid acceptor activity of a partially purified preparation of $tRNA^{Lys}$ and $tRNA^{Leu}$. The fractional amino acid acceptor activity (*13, 28*) for two concentrations of a DEAE-cellulose–purified preparation of total tRNA from HB101 cells that overproduced the $tRNA^{Phe}$ (*18*), supplemented with varying amounts of RPC-5–purified $tDNA^{Phe}$ or $tRNA^{Lys}$, was determined as described in Table 1. The data shown are mean values from six determinations, and the maximum deviation from the mean was less than 5%; Leu acceptor activity for 3 nmol (■—■) and 6 nmol (□—□) of tRNA, and Phe or Lys acceptor activity for 3 nmol (◆—◆) and 6 nmol (◇—◇) of tRNA.

tive kinetic analysis of the aminoacylation reactions for both $tDNA^{Lys}$ species, as well as a partially purified preparation of $tRNA^{Lys}$ (Fig. 5B and Table 2), are similar to and consistent with the aminoacylation kinetics observed for the $tDNA^{Phe}$ and $tRNA^{Phe}$ (Fig. 5A), since the presence of the deoxyribose similarly affected the kinetic parameters for the aminoacylation reaction by yielding a threefold increase in the observed K_m and a twofold reduction in the observed V_{max} for the dU species. Additionally, the observed V_{max} for the (dT) $tDNA^{Lys}$ was lower than that obtained for the corresponding dU species in either the presence or absence of organic solvent and polyamine.

Since the experiments presented above clearly demonstrated aminoacylation of a DNA form of a tRNA when the reaction was incubated at *p*H 5.5 rather than at *p*H 7.6, we investigated why the acidic conditions were necessary to observe tDNA aminoacylation. Although the experiments presented above do not directly address this issue, there are two possible reasons why the acidic *p*H conditions are necessary for tDNA aminoacylation. The first is that acidic *p*H alters either the tDNA or aminoacyl-tRNA synthetase solution structure, or both, such that the tDNA aminoacylation reaction can proceed more efficiently, and the second is that the acidic conditions stabilize the amino acid–tDNA ester bond. In regard to the first explanation, earlier nuclear magnetic resonance (NMR) studies (*18*) reported that at *p*H 5.5 in the presence of magnesium (the conditions used to aminoacylate the tDNAs), there is only a slight change in the proton exchange rate for the A_9-A_{23}-U_{12} tertiary structure triple in yeast $tRNA^{Phe}$, although large structural changes are observed when yeast $tRNA^{Phe}$ is incubated at *p*H 4.3 in the absence of Mg^{2+} (*19*). Also, the association between aminoacyl-tRNA synthetases and tRNAs is increased when the *p*H is reduced from 7.6 to 5.5 (*20*). Other studies revealed that subtle structural changes in the aminoacyl-tRNA synthetase at acidic *p*H may reduce the stringency of the aminoacylation reaction (*21*). For example, we have shown that several noncognate *E. coli* tRNAs could be aminoacylated with Phe by yeast Phe-tRNA synthetase at *p*H 5.8 in the presence of Mg^{2+} (*22*). Nonetheless, the tDNA inhibited aminoacylation of the corresponding tRNA at *p*H 7.6 in the presence of Mg^{2+} (Fig. 4), indicating that the tDNA structure is at least sufficiently similar to the tRNA solution structure to disrupt the interaction of the tRNA with its cognate aminoacyl-tRNA synthetase. Taken together, these observations may indicate that the tDNA solution structure is similar to that of the corresponding tRNA at both *p*H 7.6 and *p*H 5.5 in the presence of Mg^{2+}. If this conclusion is correct, then an additional factor must account for the increased tDNA aminoacylation activity observed at *p*H 5.5.

Since the aminoacyl-tRNA ester bond is often stabilized by using more acidic conditions, we suspected that a second explanation for the observed rates of tDNA aminoacylation may be that the rate of non-enzymatic deacylation at acidic *p*H is decreased. Thus we measured the *p*H-dependent nonenzymatic deacylation rates for the tDNAs and their respective tRNAs. The aminoacyl-tDNA bond is much more unstable in tris-HCl buffer at *p*H 7.6 than the corresponding aminoacyl-tRNA bond (Fig. 6), whereas both the aminoacyl-tRNA and aminoacyl-tDNA bonds show similar stability in cacodylate buffer at *p*H 5.5. We recently observed that the tDNAs can be aminoacylated at *p*H 7.6 using Hepes instead of tris buffer (*14*). Thus the instability of the aminoacyl-tDNA bond due to the tris buffer at *p*H 7.6 may account for the reduced tDNA aminoacylation rate at this *p*H and our original observation of a more stable aminoacyl-tDNA bond at *p*H 5.5 in cacodylate buffer. Based on these results we can conclude that, except for the requirement of a 2′ hydroxyl group on the 3′ terminal nucleotide of specific tRNAs (*12, 13*), the presence of the 2′ hydroxyl group in nucleotides at the remaining positions is not absolutely necessary for the tRNA aminoacylation reaction.

Table 2. Summary of the kinetic parameters for the aminoacylation of RPC-5–purified $tDNA^{Phe}$ and $tDNA^{Lys}$ and the corresponding DEAE-cellulose–purified *E. coli* tRNAs by a partially purified preparation of aminoacyl-tRNA synthetase. The results shown are the mean values from six experiments where the deviation from the mean was less than 5%, and the nucleic acid concentrations were calculated from the amino acid acceptor activity obtained under the optimal conditions (Table 1). The plus sign indicates kinetic parameter determined under the optimal conditions given in Table 1 in the presence of 20% DMSO and 2% spermidine-HCl, whereas the minus sign indicates the absence of these reagents.

Transfer nucleic acid	K_m ($M \times 10^{-7}$)		V_{max} (μmol) min^{-1} mg^{-1}	
	+	−	+	−
(dT) $tDNA^{Phe}$	2	2	0.29	0.23
(dU) $tDNA^{Phe}$	2	2	0.55	0.36
$tRNA^{Phe}$	0.66	0.66	1.00	0.55
(dT) $tDNA^{Lys}$	2.5	2.5	0.31	0.23
(dU) $tDNA^{Lys}$	2.5	2.5	0.66	0.37
$tRNA^{Lys}$	0.77	0.77	0.90	0.66

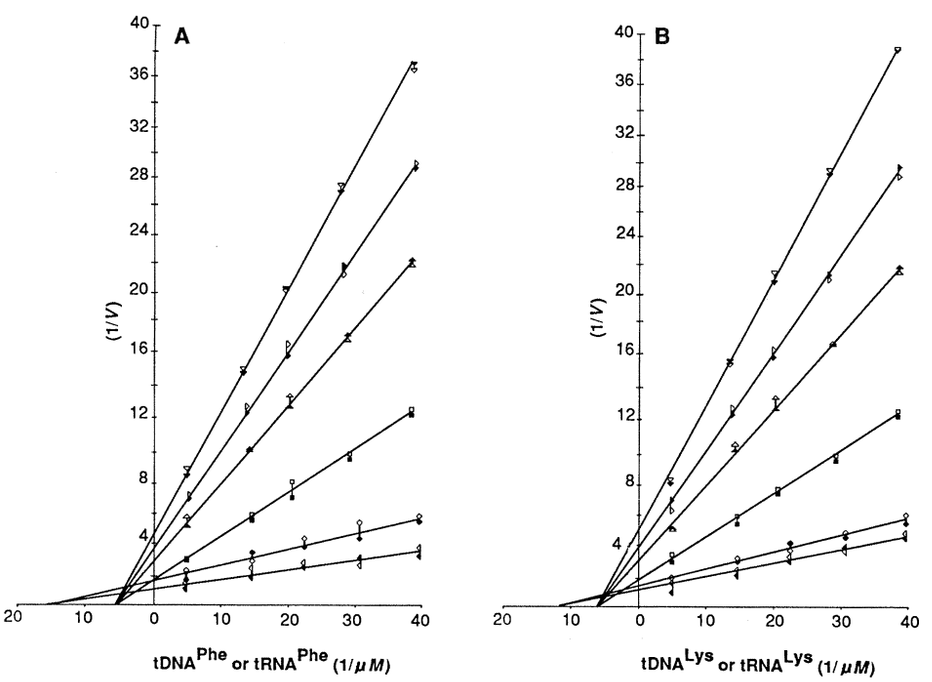

Fig. 5. Aminoacylation kinetics of purified tDNA and the respective partially purified tRNA in either the presence or absence of DMSO and spermidine for (**A**) $tDNA^{Phe}$ and (**B**) $tDNA^{Lys}$. The values for the reciprocal concentrations of the tDNA and tRNA are based on their respective amino acid acceptor activity as determined under optimal conditions (Table 1). The reciprocal velocities plotted are the extreme values from quadruplicate aminoacylation reactions incubated at 37°C for 5 min with [(△—▲), (dT) tDNA; (□—■), (dU) tDNA; and (◁—◀), tRNA] and without [(▽—▼), (dT) tDNA; (△—▲) (dU) tDNA; and (◆—◇) tRNA] 20% DMSO and 2 m*M* spermidine-HCl. The results from parallel reactions incubated in the absence of tDNA or tRNA were subtracted from the data points prior to plotting.

Since the results presented above demonstrate that aminoacylation of a DNA form of a tRNA can be aminoacylated, how can a DNA molecule attain a solution structure biologically similar to that of a tRNA such that it can be recognized by its cognate aminoacyl-tRNA synthetase? In this regard, the absence of the 2′ OH in DNA allows for a more flexible structure that can readily assume one of a number of polymorphic structural forms (*23, 24*). Thus a tDNA could assume an overall solution structure that can be aminoacylated with kinetic parameters similar to those of the corresponding tRNA. Since the deoxyribose sugar can assume either the A form with a C3′-*endo* configuration or the B form with its characteristic C2′-*endo* configuration (*23*), the deoxyribose moiety does not prevent DNA from assuming a typical RNA A-form, and the absence of the 2′ OH actually allows DNA to be much more flexible than RNA.

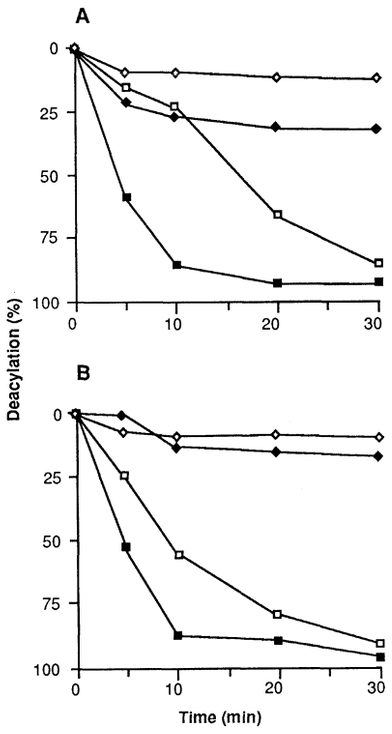

Fig. 6. Time-dependent deacylation of (**A**) $tDNA^{Phe}$ and $tRNA^{Phe}$ and (**B**) $tDNA^{Lys}$ and $tRNA^{Lys}$ at *p*H 7.6 and *p*H 5.5. The tDNAs and tRNAs were aminoacylated under the conditions described in Table 1, adjusted to *p*H 4.5, phenol extracted, and ethanol precipitated. Portions of the aminoacylated species were dissolved in either 10 m*M* tris-HCl, *p*H 7.6, or 10 m*M* cacodylate-KOH, *p*H 5.5, and immediately incubated at 37°C. At the times indicated, aliquots were removed, and the acid-precipitable radioactivity was determined (Table 1). The results plotted represent the mean values from six separate experiments and in all cases the maximum deviation from the mean was less than 5%; percent deacylation of at *p*H 7.6 of (■—■) tDNA and (□—□) tRNA; and percent deacylation of tDNA at *p*H 5.5 of (◆—◆) tDNA and (◇—◇) tRNA.

In contrast, the presence of the 2′ OH hinders RNA from assuming a conformation other than A form, by sterically destabilizing the RNA B form (*23*). In its A form, the RNA is less flexible than DNA because the 2′ OH is hydrogen bonded through water bridges with the O2 in pyrimidines and the N3 in purines. These interactions enhance the D3′-*endo* ribose pucker and the *anti* base conformation typical of the RNA A form (*23*). Thus it is more difficult for an RNA to assume the various polymorphic structures that have been observed for DNA, which is more flexible, although other RNA forms are possible (*25*). Thus our experiments demonstrating tDNA aminoacylation should not be surprising from a structural perspective, since the absence of the 2′ OH in the tDNA must allow the flexibility and polymorphism necessary to achieve the typical RNA solution structure recognized by the appropriate aminoacyl-tRNA synthetase. Although a detailed analysis of the solution structure of the synthetic tDNAs as a function of *p*H will be necessary to confirm the actual tDNA form under the conditions for optimal aminoacylation, the tDNA solution structure must be quite similar to that of the tRNA under the aminoacylation conditions studied.

The presence of dT in place of dU in the positions in the tDNAs that are usually occupied by a ribouridine or its derivatives in the respective tRNAs reduced the observed V_{max}, but had little measurable effect on the observed K_m. Increased base-stacking interactions in the (dT) tDNAs may make the conformation necessary for aminoacyl-tDNA bond formation less favorable. Alternatively, the presence of the additional methyl group on the uridine residues may introduce additional steric constraints that affect only the observed V_{max} of aminoacylation. Furthermore, the observed K_m for the aminoacylation reaction for the tDNAs in either the presence or absence of DMSO and spermidine is similar to but one-half to one-third that of the observed K_m for the corresponding tRNA. These observations suggest that the major effect of the sugar is to alter the K_m, whereas base modification of dU to dT has its major effect by altering the V_{max} of the aminoacylation reaction, an effect similar to that observed in the presence of organic solvent. Previous studies have demonstrated that specific tRNA base modifications similarly affected the V_{max} but not the K_m obtained for two tRNA-dependent reactions (*3*). These results are consistent if the presence of the ribose or deoxyribose backbone in the tRNA allows for differences in the overall structure that reflect conformational changes affecting the aminoacyl-tRNA binding step in the aminoacylation reaction, whereas the presence of dT and dU, or the organic solvent and polyamine, or both, alter the interactions that lead to product formation, that is, the aminoacyl-tRNA bond.

Finally, although we anticipate determining if the synthesized tDNAs will bind to bacterial initiation factors and bacterial ribosomes, as well as if the tDNAs can fulfill the adaptor function in an in vitro protein synthesis system (*26*), at present we only can speculate on the results of these experiments. Since the most stringent step in protein synthesis is that of aminoacylation (*27*) and the tDNAs can be aminoacylated, the aminoacylated form of these tDNAs may function in these reactions. Nonetheless, DNA analogs of at least two tRNAs can be aminoacylated, and these tDNAs retain the structural parameters necessary for recognition by their cognate aminoacyl-tRNA synthetases while still containing the structure necessary for discrimination by noncognate aminoacyl-tRNA synthetases.

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Induction of Gene Amplification by Arsenic

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Arsenic is a well-established carcinogen in humans, but there is little evidence for its carcinogenicity in animals and it is inactive as an initiator or tumor promoter in two-stage models of carcinogenicity in mice. Two arsenic salts (sodium arsenite and sodium arsenate) induced a high frequency of methotrexate-resistant 3T6 cells, which were shown to have amplified copies of the dihydrofolate reductase gene. The ability of arsenic to induce gene amplification may relate to its carcinogenic effects in humans since amplification of oncogenes is observed in many human tumors. The inability of arsenic to induce gene mutations may relate to the negative results of arsenic in long-term animal studies and suggests that these experiments may not detect some environmental agents that act late in the carcinogenic process in humans.

ARSENIC IS AN UNUSUAL ENVIRONmental substance in terms of its carcinogenic response in humans and animal models. Exposure of humans to inorganic arsenic compounds in drugs, drinking water, and occupational environments is associated with increased risks of skin cancer, lung cancer, and possibly liver cancer (*1, 2*). However, little evidence exists for the carcinogenicity of arsenic to animals (*2–5*). Yet, sodium arsenite and sodium arsenate reproducibly induce morphological transformation of rodent cells in culture (*6, 7*). A distinction does exist, however, between arsenic and most chemicals that induce cell transformation in that arsenic is inactive in inducing gene mutations at specific genetic loci (*7*). An elucidation of the mechanism of arsenic-induced cellular changes may help in better understanding human carcinogenesis and the relationship between carcinogen-induced events in humans and in rodents. The use of long-term rodent carcinogenicity studies as assays for detecting potential human carcinogens requires an understanding of the apparent false negative results in the animal cancer studies.

In this report, we present evidence that sodium arsenite and sodium arsenate are potent enhancers of amplification of the dihydrofolate reductase (DHFR) gene in mouse 3T6 cells. Amplification of the DHFR gene was measured in mouse 3T6 cells by selecting cells that form colonies in the presence of methotrexate. Cells with an increased copy number of the DHFR gene have increased levels of DHFR enzyme and are resistant to methotrexate (MTX^R) (*8–10*). Treatment of mouse 3T6 cells with sodium arsenite or sodium arsenate induced dose-dependent increases in the number of MTX^R colonies (Fig. 1). Sodium arsenite was active at a lower concentration than sodium arsenate. This is the same relative potency of the two compounds in cell transformation assays (*7*), and the concentration ranges for induction of gene amplification and cell transformation are the same. Sodium arsenite has been noted to be more active than sodium arsenate in other biological assays, and it has been suggested that this is due to differences in the uptake of trivalent versus pentavalent arsenic (*11*). Sodium arsenite and sodium arsenate induced MTX^R colonies at MTX concentrations of 150 to 300 n*M* (Fig. 2). To test for a possible interaction between methotrexate and arsenic, cells were grown in lower doses of methotrexate and arsenic and no increased viability was observed.

The surviving MTX^R 3T6 colonies were isolated, grown in culture and shown to be resistant to MTX and to have amplified DHFR genes. The copy number of the DHFR gene was estimated by comparing the extent of hybridization by dot blot analysis of radiolabeled cloned DHFR cDNA or actin gene to DNA isolated from parental 3T6 cells and MTX^R clones (*8, 10*). Approximately 50% (9/17) of the MTX^R clones induced by arsenic (0.2 to 0.8 μg/ml) had amplified copy numbers of the DHFR gene ranging from 2- to 11-fold, which is consistent with the findings of others (*9, 10*).

Table 1. Effect of sodium arsenate and sodium arsenite on MTX^R colonies.

Treatment*	Relative survival (%)	MTX^R colonies/ 5×10^5 cells†	MTX^R colonies/ 5×10^5 surviving cells
None	100	0.7 ± 0.6	0.7
Sodium arsenite (μ*M*)			
0.2	100	2 ± 1.5	2
0.4	100	5 ± 2	5
0.8	115	7 ± 3	6
1.6	45	17 ± 3	38
3.1	15	33 ± 5	226
6.2	1	82 ± 14	8200
Sodium arsenate (μ*M*)			
1	100	2 ± 1	2
2	100	4 ± 1	4
4	100	13 ± 6	13
8	40	23 ± 9	57
16	11	62 ± 13	569
32	1	161 ± 27	16000

*0.2 μ*M* sodium arsenate is equivalent to 0.025 μg/ml; 1 μ*M* sodium arsenate is equivalent to 0.313 μg/ml. †Colonies selected in 200 n*M* MTX for 21 days (±SD).

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