ments it is reasonably certain that antiserum to LDH-X acts primarily by interfering with implantation or causing abortion (or both) after implantation occurs. Whether the antiserum affects the fertilized ovum directly, for example, by causing embryonic failure, or whether it induces the creation of a hostile endometrial environment (or both) remains an open question.

We also ascertained that the antiserum action was temporary, at least after the 1- to 4-day regimen of treatment. Seven mice of an AS group were mated 21 days after their last injection, and all of them delivered normal litters. In addition, the serum injections had no obvious deleterious effects on the mice in terms of weight loss or feeding behavior, nor were there any gross pathological lesions detectable from postmortem examination of viscera.

There is no detectable LDH-X in the female, nor is there any associated with the female reproductive system. We have been unable to detect by immunological tests nonspecific antigens in extracts of various portions of the female reproductive tract which react with the antiserum. Nevertheless, precedents for this phenomenon exist. As was noted above, there is a long history of efforts to control fertility of females immunologically. Of the more recent work, McLaren (*3*) and Bell (*4*) reported fertility reduction in female mice immunized with homologous spermatozoa or testis homogenates. Similarly, infertility has been induced immunologically in cattle (*5*), guinea pigs (*6*), and rabbits (*7*). Bell and McLaren (*8*) found that a subcellular fraction of spermatozoa, the 1200*g* supernatant of disrupted cells, depressed fertility in female mice. Their work indicated that whole spermatozoa were not required for antigenicity and that reduced fecundity was due to immunological impairment of the fertilization process.

Passive immunization causing fertility depression in female mice was reported by Sadri *et al.* (*9*). In this study antiserum induced in rabbits against a saline extract of mouse testes completely inhibited pregnancy in females injected on days 1 to 4 or 6 to 8 after mating, presumably by interfering with implantation. Treatment of two-cell embryos from the rabbit with bovine antiserum against rabbit semen did not reduce their survival rate (*10*). Such antiserum treatment did, however, have a detrimental effect on blastocyst survival (*11*).

Apparently, this is the first report of the effect of an antibody to a specific enzyme on reproductive processes. The data clearly illustrate that this antibody to LDH-X can disrupt pregnancy. Most intriguing is the fact that the enzyme is found only in the male reproductive system while the antiserum to it can act in the female. How this occurs is of course a compelling question of immediate significance to an understanding of the normal reproductive process. Also, the prospect of exploiting this finding in a fertility control program does exist.

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12. This work was supported by a contract with the National Institute of Child Health and Human Development.

10 February 1972

Nucleotide Sequence of a Lysine Transfer Ribonucleic Acid from Bakers' Yeast

Abstract. *The nucleotide sequence of one of the two major lysine transfer RNA's from bakers' yeast has been determined. Its structure is compared to that of a lysine tRNA from a haploid yeast. A total of 21 nucleotides differ in the two molecules. Only the T-ψ-C-G (thymidine-pseudouridine-cytidine-guanosine) loop and its supporting stem are identical.*

The nucleotide sequence of a major $tRNA^{Lys}$ (*1*) from bakers' yeast has been worked out. Its sequence is shown in Fig. 1A in the cloverleaf arrangement. The $tRNA^{Lys}$ was purified from bakers' yeast tRNA (*2*) by countercurrent distribution in an ammonium sulfate system (*3*). The faster moving $tRNA^{Lys}$ was further purified by reverse-phase chromatography with tricaprylmethylammonium chloride (*4*).

The fragments produced by ribonuclease T1 and pancreatic ribonuclease are shown in Fig. 2. Their nucleotide sequences were determined by the specificity of the nuclease used, digestion of pancreatic ribonuclease fragments with ribonuclease T1, digestion of ribonuclease T1 fragments with pancreatic ribonuclease, partial hydrolysis with snake venom phosphodiesterase (*5*), and polynucleotide phosphorylase (*6*). The large fragments isolated after incomplete digestion with pancreatic ribonuclease and ribonuclease T1 in the absence of Mg^{2+} are shown in Fig. 2. These large fragments were analyzed by complete digestion with ribonuclease T1 or pancreatic ribonuclease.

Seventeen of the 76 nucleotides in the $tRNA^{Lys}$ are modified; only the rat liver $tRNA^{Ser}$ (*7*) and the torula yeast $tRNA^{Tyr}$ (*8*) are as highly modified. The $tRNA^{Lys}$ has five pseudouridine residues, including a 5′-terminal pψp. Although Gray and Lane (*9*) found that 4 percent of mixed bakers' yeast tRNA contained pψp, this is the first time that pψp has been found in a purified tRNA.

The anticodon is S-U-U or Z-U-U. The structure of S has been determined by Baczynskyj *et al.* (*10*) to be 2-thio-5-carboxymethyluridine methyl ester. The alternate nucleotide (Z) is probably a derivative or a degradation product of S, since Z and S were both converted to 5-carboxymethyluridine by desulfurization with cyanogen bromide (*11*). Z is probably an oxidation product of S, as treatment with iodine converted S into a product whose spectral properties were very similar to those of Z. The dinucleotide ZpUp does not have a sufficient number of negative charges for Z to be a symmetrical disulfide. It could be a mixed disulfide formed with an unknown sul-

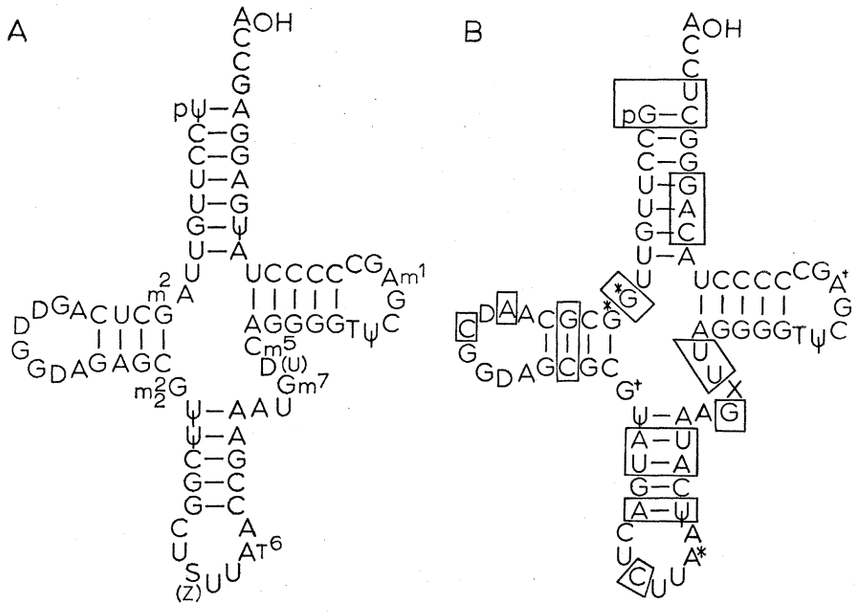

Fig. 1. Cloverleaf configurations of bakers' yeast $tRNA^{Lys}$ (A). Haploid *Saccharomyces cerevisiae* αS288c $tRNA^{Lys}$ (B) sequenced Smith *et al.* (*18*). Solid lines surround the nucleotides that differ from those in Fig. 1A.

fur compound at some stage in the isolation.

Three of the products of partial digestion with pancreatic ribonuclease in Fig. 2 include the intact anticodon loop. This seems unusual since there are so many pyrimidines in this region. These results suggest that in $tRNA^{Lys}$ the anticodon loop is in a protected conformation, in contrast to most tRNA's where this loop is digested first under partial digestion conditions.

In addition to these unusual properties, this tRNA also exhibits anomalous coding properties. Derivatives of 2-thiouridine have been found in the G-A-A codon specific $tRNA^{Glu}$ from yeast (*12*) and *Escherichia coli* (*13*), and in these cases it has been suggested that the 2-thiouridine is unable to form a wobble base pair with the final G in the G-A-G codon. By analogy, our $tRNA^{Lys}$ should code with A-A-A, but not A-A-G. However, our purified $tRNA^{Lys}$ was found by Woodward and Herbert (*14*) to be able to transfer lysine into all the lysine positions in hemoglobin when tested in a cell-free reticulocyte system. So it appears to respond to both of the lysine codons: A-A-A and A-A-G. This is what would be expected from the wobble hypothesis (*15*) if S (or Z) is functioning like U. In apparent contradiction to these results, Mitra *et al.* (*16*) have found that both of the major $tRNA^{Lys}$ peaks from brewers' yeast were bound to ribosomes in the presence of A-A-G, but not when A-A-A was added. Further work will be required to clarify the coding properties of yeast $tRNA^{Lys}$.

Dudock *et al.* (*17*) have suggested that the stem of the dihydrouridine loop (the double-stranded region supporting the loop containing D) is the recognition site for yeast phenylalanyl-tRNA synthetase. This enzyme can attach phenylalanine to $tRNA^{Phe}$ from yeast, wheat, and *E. coli*, $tRNA_1^{Val}$ (*E. coli*), and $tRNA_1^{Ala}$ (*E. coli*); all of these very likely have the sequence

-CUCGA-
-GAGC-

in the stem of the dihydrouridine loop. Our $tRNA^{Lys}$ has the same sequence. This is the first time these nucleotides have been found in the dihydrouridine stem in another yeast tRNA. Our $tRNA^{Lys}$ also has the 7-methylguanine in the minor loop that the others have. Since the $tRNA^{Lys}$ does not accept phenylalanine, these two sites cannot be the specificity site for yeast phenylalanyl-tRNA synthetase.

Figure 1B shows the $tRNA^{Lys}$ from a haploid yeast whose nucleotide sequence has been determined by Smith *et al.* (*18*). The bakers' yeast $tRNA^{Lys}$ that we have not purified codes for A-A-G, and therefore it is probably comparable to the $tRNA^{Lys}$ sequenced by Smith *et al.* The nucleotides that differ from those in the tRNA which we have sequenced are shown within solid lines. There are 21 positions where different nucleotides are found, and 13 of these are in the double-stranded portion of the cloverleaf. Only the G-T-ψ-C loop and stem are identical. The two yeast lysine tRNA's have the same number of nucleotides as do many of the other isoaccepting tRNA's whose structures are known. Since there are so many differences in the individual nucleotides in many of the other isoaccepting tRNA's as well as in the lysine tRNA's, the overall size and shape of the tRNA molecule is probably important in its recognition by the aminoacyl-tRNA synthetase. What parts of the nucleotide sequence are important in recognition by the synthetase are unknown. The anticodon stem and the minor loop are probably not important recognition sites, since

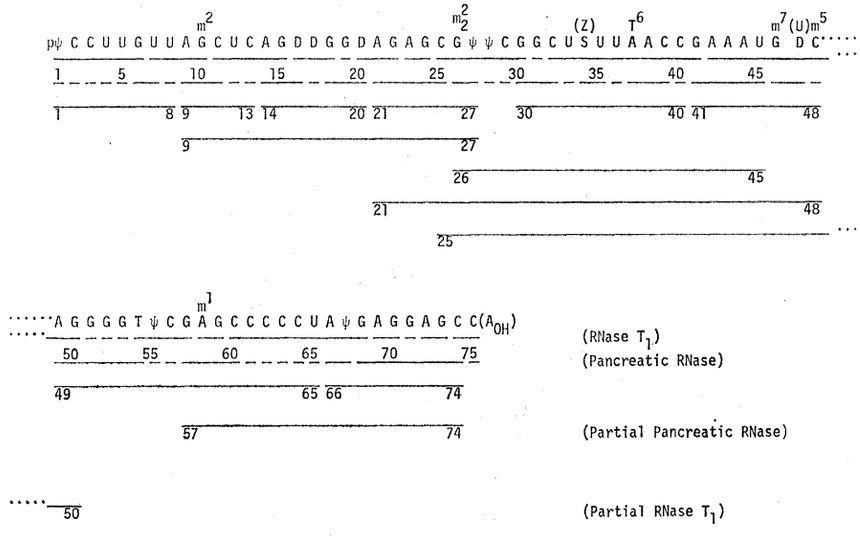

Fig. 2. Fragments used to establish the nucleotide sequence of bakers' yeast $tRNA^{Lys}$.

they are so different in the two lysine tRNA's. It is interesting that the sequence C-C-U-U-G-U-U near the 5′-terminus is preserved even though three changes have occurred in the nucleotides on the other side of the stem.

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References and Notes

1. Abbreviations used: tRNA, transfer ribonucleic acid; A, adenosine; C, cytidine; G, guanosine; U, uridine; p or -, phosphate residue, on the left of the nucleoside symbol a 5′-phosphate and on the right a 3′-phosphate; ψ, pseudouridine; T, ribothymidine; m, methyl group whose position is indicated by the superscript; m_2^2G, N^2,N^2-dimethylguanosine; D, dihydrouridine; (U), uridine makes up about 25 percent of the nucleosides at this position; S, 2-thio-5-carboxymethyluridine methyl ester; Z, derivative or degradation product of S; T^6A, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine; X, unidentified nucleoside; A* and A^+, adenosine derivatives; G* and G^+, guanosine derivatives; Phe, Val, Gly, Glu, Lys, Ser used as superscripts indicate tRNA specific for phenylalanine, valine, glycine, glutamic acid, lysine, and serine, respectively.
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17 January 1972; revised 22 February 1972

Unilateral Increase of Auditory Sensitivity following Early Auditory Exposure

Abstract. *A 30-second exposure (priming) to a loud noise induced a long-lasting susceptibility to audiogenic seizures and a 15-decibel decrease in threshold to the Preyer acoustic startle reflex in C57BL/6J mice. Both effects were absent when the subjects were primed in one ear and subsequently tested in the contralateral ear. It was postulated that the primary effect of priming is an increase in auditory sensitivity due to changes in the ear itself or in those parts of the auditory system which receive their input exclusively from one ear.*

This experiment investigated the long-term effects of a brief, high-intensity auditory exposure on subsequent sensitivity to high-intensity sounds. The data suggest that the initial 30-second exposure resulted in a 15-db increase of sensitivity after 5 days which could be restricted to either the right or left auditory pathway.

Inbred mice of the C57BL/6J and SJL/J strains are normally resistant to sound-induced convulsions (*1*). However, a 30-second exposure to a loud noise during a sensitive period of development (*2*) can render both strains highly susceptible to the audiogenic seizure syndrome (*3*). Fuller and Collins (*4*) devised an ingenious technique whereby this effect was confined to a single ear of the SJL/J mouse by having the other ear temporarily plugged with glycerine during priming and subsequent testings. The present experiment used this technique to demonstrate that an independent measure of auditory sensitivity—the Preyer acoustic startle reflex (*5*)—shows an identical response.

Fifty inbred mice of the C57BL/6J strain were acoustically primed in either the right ($N = 25$) or the left ($N = 25$) ear at 16 (± ½) days of age. This was accomplished by flooding one ear with glycerine and placing the subject in a cylindrical glass jar (30 by 30 cm). An electric bell was then mounted atop the container, and the subjects were exposed to 30 seconds of 110 db (absolute) (in reference to 2×10^{-4} dyne/cm^2) noise. None of the subjects exhibited audiogenic seizures during this acoustic priming exposure. The acoustically primed ear was then flooded with glycerine to compensate for the effects of glycerine per se.

At 21 days of age, 30 mice were reexposed to 30 seconds of bell ringing. In 15 subjects, the same ear was plugged as during priming (ipsilateral condition), while another 15 mice were tested with glycerine in the opposite ear (contralateral condition). The incidence of audiogenic seizures was observed in these mice. Similarly, another 20 subjects were tested for threshold response to the Preyer reflex with glycerine blocking either the ipsilateral or the contralateral ear. These mice were successively exposed to a series of shaped (10 msec rise and decay time) bursts of 15-khz pure tones of 1 second duration. The equipment has been described elsewhere (*6*). The stimulus intensity was increased in increments of 2 db (method of ascending limits) to ascertain the threshold of the Preyer reflex, defined as the minimum intensity of sound that elicited a reflexive ear movement 50 percent of the time in each subject.

Fuller and Collins' ipsilateral effect of priming of audiogenic seizures in SJL/J mice was replicated with C57-BL/6J mice in this study. All of the 15 ipsilateral subjects exhibited an audiogenic seizure, with an average latency of 4.2 seconds. Fourteen of the 15 contralateral subjects failed to convulse (Fisher's exact probability test, P ipsilateral > contralateral $= 1.33 \times 10^{-8}$).

The thresholds to the Preyer reflex exhibited an equally extreme effect. Those ten ipsilaterally tested subjects had a mean threshold of 73.8 db, while the ten contralaterals had a mean threshold of 89.2 db. There was no overlap between the distributions (range of 68 to 80 for ipsilaterals; 86 to 92 for contralaterals, $t = 12.3$; d.f. $= 18$; P ipsilateral > contralateral << .0001).

The effects of acoustic priming on audiogenic seizures could be explained in a variety of ways: a lowered threshold to the propagation of epileptiform activity in neural structures which are associated with auditory pathways, creation of an epileptic focus in auditory or associated regions, or a lowered auditory threshold. The Preyer reflex data suggest that the latter interpretation is more likely correct. This reflex has widely been used as an index of auditory sensitivity. Although the correlation is not invariable, it typically parallels the curves for auditory nerve–evoked responses and for cochlear