12, and Fig. 2, lanes 16 and 19; nt 57, 58, 63, and 64) occurs mainly at thymines that lie at either end of a $(dT)_6 \cdot (dA)_6$ sequence. Because oligo[(dA)·(dT)] sequences can adopt a non-B structure having a high propeller twist (P-DNA), there is rather poor stacking at junctions between B-DNA and P-DNA regions (*31, 32*). Hence the helix may "buckle" under high torsional strain at these sites, allowing accessibility to OsO_4.

While most probes yield similar results at *p*H 5 and 6, hydroxylamine exhibits at *p*H 6 a moderate reactivity over most of the homopyrimidine sequence which is superimposed upon the localized hyperreactivity seen at *p*H 5 (Fig. 1a, lanes 7 and 8, and Fig. 1b, lane 4). Because the hydroxylamine reaction at *p*H 6 takes place under conditions of high salt (*10*) and nearly neutral *p*H, both of which somewhat disfavor the altered structure (*6, 7*), there may be an equilibrium mixture of B-DNA, the altered conformation, and intermediate structures which are substantially unpaired and therefore reactive over much of their length. It is not clear why this anomalous reactivity at *p*H is seen with hydroxylamine but not with methoxylamine.

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Changing the Acceptor Identity of a Transfer RNA by Altering Nucleotides in a "Variable Pocket"

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The specificity of $tRNA^{Arg}$ (arginine transfer RNA) for aminoacylation (its acceptor identity) were first identified by computer analysis and then examined with amber suppressor tRNAs in *Escherichia coli*. On replacing two nucleotides in $tRNA^{Phe}$ (phenylalanine transfer RNA) with the corresponding nucleotides from $tRNA^{Arg}$, the acceptor identity of the resulting tRNA was changed to that of $tRNA^{Arg}$. The nucleotides used in the identity transformation occupy a "variable pocket" structure on the surface of the tRNA molecule where two single-stranded loop segments interact. The middle nucleotide in the anticodon also probably contributes to the interaction, since an amber suppressor of $tRNA^{Arg}$ had an acceptor identity for lysine as well as arginine.

TRANSLATION OF THE GENETIC CODE by tRNA molecules requires two specificities, one for amino acids and the other for nucleotide sequences. The specificity of a tRNA for an amino acid is referred to as its acceptor identity (*1*). The acceptor identity is read by 1 of 20 aminoacyl-tRNA synthetase enzymes, which attach the proper amino acid only to its cognate tRNA species. Computer algorithms can effectively locate the nucleotides in tRNA that determine acceptor identity (*2, 3*).

The acceptor identity of a tRNA results from two types of interactions, the productive interaction of the tRNA with its cognate aminoacyl-tRNA synthetase and the nonproductive interaction of the tRNA with all other aminoacyl-tRNA synthetases (*4, 5*). The acceptor identity of tRNA appears to be determined by multiple nucleotides whose locations are not conserved between different molecules. This plurality may sharpen aminoacyl-tRNA synthetase

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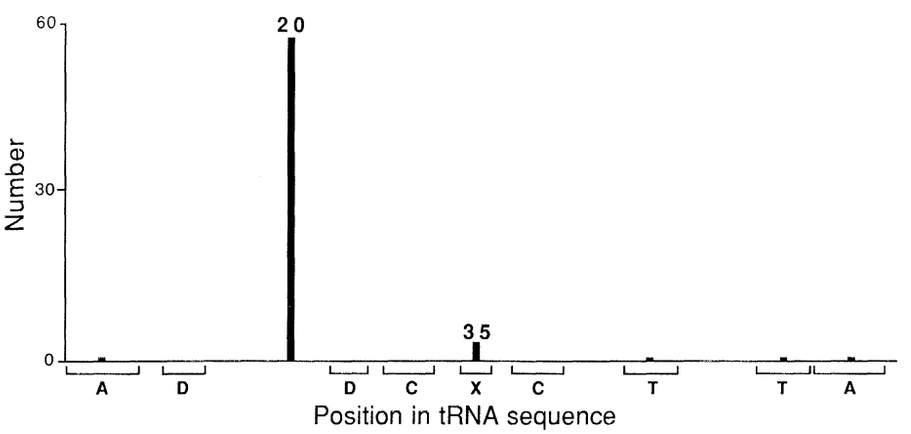

Fig. 1. Frequency of positions in discriminators of $tRNA^{Arg}$ (*2*). Frequency on the vertical axis is a function of position in the tRNA on the horizontal axis. Cloverleaf stem segments on the horizontal axis are indicated as A, acceptor; D, dihydrouridine; C, anticodon (X marks the anticodon); and T, TΨ. The data set contained 65 tRNA sequences. There were 58 one-position discriminators involving position 20 and four two-position discriminators involving positions 4 and 35; 35 and 51; 35 and 63; and 35 and 69.

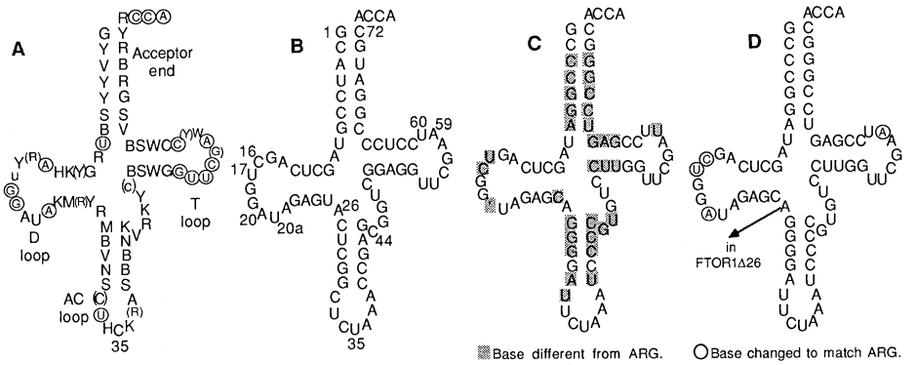

Fig. 2. (**A**) Composite of six *E. coli* $tRNA^{Arg}$ sequences and sequences of amber suppressors of (**B**) $tRNA^{Arg}$, (**C**) $tRNA^{Phe}$, and (**D**) the FTOR1 tRNA. In the composite, the nucleotides that are constant in all *E. coli* tRNAs are circled and those that are constant purine or pyrimidine residues are in parentheses. Abbreviations for combinations of variable nucleotides are Y = C, U; V = A, C, G; S = C, G; B = C, G, U; R = A, G; K = G, U; H = A, C, U; M = A, C; W = A, U; and N = all. Lowercase letters (positions 17 and 48) indicate that the combination contains a deletion. The symbol ′ indicates an alignment gap in PHE and a deletion in FTOR1Δ26. The nucleotide sequence of suppressor ARG is based on the sequence of $tRNA^{Arg}_{ACG-1}$ (*18*). The shaded nucleotides in PHE are different from those in ARG. The circles in FTOR1 indicate where the nucleotides in PHE were changed to match ARG. Genes encoding the amber suppressor tRNAs were synthesized at the University of Wisconsin Biotechnology Center, ligated into plasmid pGFIB, and cloned in *E. coli* XAC/A16 (*9*).

discrimination between different tRNAs.

The nucleotides responsible for acceptor identity could logically be confined to the anticodon sequence, and some tRNAs primarily use this site (*6*). However, other tRNAs with altered anticodons corresponding to nonsense suppressors retain their acceptor identity (*7*), indicating that the primary nucleotides for acceptor identity reside elsewhere in these tRNAs, for example, in a patch on the surface of the molecule where two single-stranded loop segments interact. The latter region, termed the variable pocket (*8*), contributes to the acceptor identities of $tRNA^{Phe}$ (*9, 10*) and $tRNA^{Ala}$ (*11*).

The acceptor identity of nonsense suppressor tRNAs has been studied in vivo by genetic means (*1, 5, 9, 11–13*). This approach has the advantage of reflecting the net outcome of 20 different aminoacyl-tRNA synthetase enzymes competing for the suppressor tRNA under physiological conditions. The effect of overproducing the suppressor tRNA in these systems has yet to be determined and may prove to be a limitation. Other approaches to acceptor identity have relied on measurements of the kinetics of tRNA aminoacylation in vitro (*6, 10*) and on structural studies of cocrystals of tRNAs and aminoacyl-tRNA synthetases (*14, 15*). A combination of approaches is needed to obtain a comprehensive understanding of the molecular basis of tRNA identity.

To study the acceptor identity of *Escherichia coli* $tRNA^{Arg}$, we compared tRNA sequences by computer analysis (*2, 3*) to predict which nucleotide residues might transform the acceptor identity of another tRNA to that of $tRNA^{Arg}$. These results were used to design synthetic genes of amber suppressor tRNAs that were subsequently manufactured and functionally characterized in *E. coli*.

Cross-validation of an initial set of 66 *Escherichia coli* tRNA sequences (*16*) containing seven isoacceptors of $tRNA^{Arg}$ revealed an instability attributable to the sequence of $tRNA^{Arg}_{ACG-2}$ (*17*). The instability was manifested by obtaining 59 new discriminators for $tRNA^{Arg}$ when the sequence of $tRNA^{Arg}_{ACG-2}$ was omitted; in contrast, no more than two new discriminators accompanied the systematic elimination of any other $tRNA^{Arg}$ sequence. With its accuracy in question, the $tRNA^{Arg}_{ACG-2}$ sequence was deleted from the data set. Subsequent computer analysis (*2*) of the remaining 65 tRNA sequences disclosed that nucleotide A at position 20 (A20) is present in all six $tRNA^{Arg}$ isoacceptors and in no other tRNAs, and that four dinucleotide combinations are also distinguishing characteristics of $tRNA^{Arg}$ (Fig. 1). Each of the dinucleotide combinations included position C35, which is present in all $tRNA^{Arg}$ isoacceptors. The location of A20 and C35 in the cloverleaf structure of $tRNA^{Arg}$ is shown in Fig. 2.

The contribution of nucleotide A20 to the acceptor identity of $tRNA^{Arg}$ was examined by inserting this nucleotide in an amber suppressor gene of $tRNA^{Phe}$; the resulting variant was termed PHEA20U20a. This tRNA was used because its acceptor identity involves U20 (*9*), so the insertion of the extra nucleotide A20 would shift U20 to U20a and degrade $tRNA^{Phe}$ identity, thereby facilitating the desired transformation. However, PHEA20U20a was inactive (Table 1) and accumulated precursor RNA at the expense of the mature tRNA (Fig. 3). This result suggested that the insertion of the extra nucleotide in $tRNA^{Phe}$ caused a structural defect in the precursor RNA and poor ribonuclease P cleavage, resulting in little production of mature tRNA. The RNA may also be sensitive to nonspecific degradation.

We attempted to repair the defect in PHEA20U20a by mimicking the nucleotide environment around residue A20 of $tRNA^{Arg}$. The tertiary structure of tRNA shows that the nucleotides near residue 20 occupy a variable pocket (*8*) and include, in addition to residue 20, residues 16, 17, 20a,

Table 1. Functional properties of suppressor tRNAs. Genes for suppressor tRNAs were made from synthetic oligonucleotides. Dihydrofolate reductase protein was purified and sequenced through residue 15. The percentage of each amino acid at residue 10 was calculated; values <5% are not reported and Cys was not determined. The values for ARG are the average of two determinations, with the individual values ±9% of the average. A second determination of FTOR1 gave Arg, 78%, and Thr, 6%. A failure precluded determination of Gly in ARGU20. Suppression efficiencies were determined for amber alleles A16 and A30 in the *lacI-Z* system for cells grown at 37°C. The values are the average of triplicate measurements and are reported as the percentage of the wild-type *lacI-Z*40 fusion, which averaged 156 units. The values were corrected for first-order decay of activity, which had a half-life of 877 min for wild-type *lacI-Z*40; this correction affected the values ≤1%. The values for cells without a suppressor gene were A16, 0.000%, and A30, 0.915%. The methods and the data for PHE have been described (*9*).

Suppressor gene	Amino acid in suppressed protein (%)	Suppression (%) efficiency	
		A16	A30
ARG	Arg, 37; Lys, 55	30	62
ARGU20	Lys, 91	12	28
ARGU59	Arg, 38; Lys, 50	29	59
PHE	Phe, 86	11	22
FTOR1	Arg, 75; Lys, 5; Tyr, 6	3	16
FTOR1Δ26	Arg, 92	3	20
PHEA20U20a		0.026	0.407
PHEA20U20aA59	Arg, 72; Lys, 6; Thr, 16	3	11
PHEA59	Phe, 91; Leu, 7	4	13

59, and 60 (Fig. 4). To generate the sequence found in the variable pocket of $tRNA^{Arg}_{ACG-1}$ (*18*) in $tRNA^{Phe}$, nucleotide substitutions were made at positions 16, 17, and 59 to complement the A20 insertion; the resulting suppressor tRNA was termed FTOR1 (Fig. 2) (F, Phe; R, Arg). These alterations resulted in the appearance of mature tRNA (Fig. 3).

The acceptor identity of the FTOR1 tRNA was substantially that of $tRNA^{Arg}$, whereas the efficiency of suppression was similar to the $tRNA^{Phe}$ suppressor (Table 1). Acceptor identity was established by sequencing suppressed dihydrofolate reductase protein (*1*), which gave Arg and minor amounts of Lys and Tyr at the residue corresponding to the amber mutation; Phe was absent. The presence of amino acids other than Arg indicates that an element of the acceptor identity of $tRNA^{Arg}$ is missing in the FTOR1 tRNA.

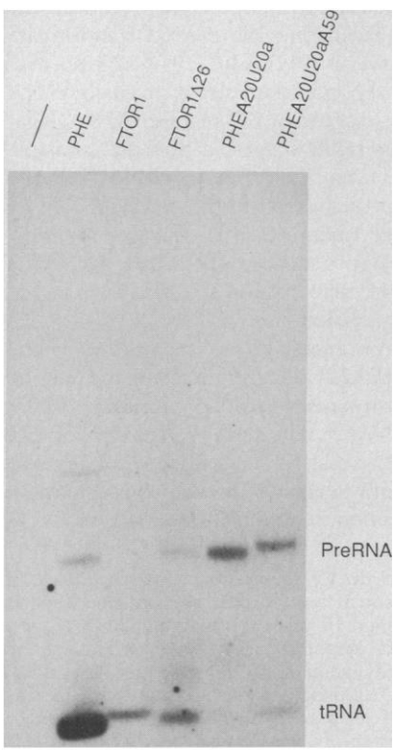

Fig. 3. RNA blot analysis (*9*). Cells carrying plasmid pGFIB containing the indicated tRNA gene insert (or none, "−") were grown in LB broth plus ampicillin at 37°C overnight, then diluted 50-fold into fresh medium. When the cell density reached 3×10^8 per milliliter, phenol was added and the RNA was isolated. RNA samples corresponding to 0.5-ml cultures were fractionated by electrophoresis on a 10% polyacrylamide gel containing tris-borate-EDTA, *p*H 8.26, transferred by electroblot to GeneScreen and probed with a ^{32}P-labeled oligonucleotide complementary to positions 33 to 50 of the amber suppressor $tRNA^{Phe}$ of *E. coli*. The probe is not complementary to positions 34 and 35 of wild-type $tRNA^{Phe}$ and hybridizes poorly with it (for example, left lane, "−"). The mobilities were precursor RNA, 11.2 cm, and tRNA, 15.7 cm.

A derivative of $tRNA^{Phe}$ containing only the A20 insertion and a U to A substitution at position 59 was constructed. This variant, PHEA20U20aA59, had an acceptor identity and efficiency similar to that of the FTOR1 tRNA (Table 1). Two observations suggest a structural rather than an acceptor identity role for A59 in PHEA20U20aA59. First, more tRNA was obtained when A59 was present (compare PHEA20U20aA59 with PHEA20U20a, Fig. 3), and, second, different isoacceptors of $tRNA^{Arg}$ contain either A or U at position 59 (Fig. 2). The proposed role for A59 may involve stabilization of the tRNA structure. The insertion of A20 in the D loop of the $tRNA^{Phe}$ molecule as in variant PHEA20U20a may destabilize the interaction of the T and D loops, which is known to be important for tRNA synthesis (*19*). When A59 rather than U59 stacks on residue G15 in the D loop (*8*), a greater stability of tRNA structure may result. The stabilization of the T and D loop interaction by a nucleotide substitution in the T loop has been reported elsewhere for phage T4 $tRNA^{Ser}$ (*20*).

Although the alteration of position 20 from A to U did not affect the amount of mature tRNA produced, both the suppression efficiency and acceptor identity were degraded in the ARGU20 suppressor tRNA (Table 1), indicating the loss of a productive interaction with the Arg-tRNA synthetase.

The FTOR1Δ26 tRNA is a nonprogrammed variant (*21*) of FTOR1 that carries a deletion of the nucleotide at position 26 (Fig. 2). Because residues 26 and 44 form a base pair in tRNA structures (Fig. 4), it was surprising that the FTOR1Δ26 tRNA was synthesized (Fig. 3) and that it retained its suppression efficiency (Table 1). This finding contrasts with the large reduction in tRNA synthesis and function observed when nucleotide 26 is deleted from a suppressor of phage T4 $tRNA^{Ser}$ (*20*). The most remarkable aspect of FTOR1Δ26, however, is that it inserts only Arg into protein, whereas the FTOR1 tRNA without the deletion also inserts other amino acids.

We have considered two possibilities to explain the enhanced acceptor identity of the FTOR1Δ26 tRNA. A structural adjustment in the tRNA molecule accompanying the nucleotide deletion is implicated in both possibilities. First, the deletion of nucleotide 26 may act negatively by fostering poorer interactions between the tRNA and the aminoacyl-tRNA synthetases corresponding to Lys and Tyr (but not Arg), which are inserted by the FTOR1 tRNA (Table 1). Alternatively, the deletion of nucleotide 26 may foster a better interaction between the tRNA and Arg-tRNA synthetase. The structural adjustment in the FTOR1Δ26 tRNA may allow the Arg-tRNA synthetase to simultaneously use A20 and an analogue of C35 through a shift in the spatial location of nucleotide C34 toward the location of position 35. Position 35 is a C in wild-type $tRNA^{Arg}$ (Fig. 2) and probably contributes to its acceptor identity.

The wild-type anticodon is apparently important for the Arg-tRNA synthetase, as the starting amber suppressor of $tRNA^{Arg}$ had a heterogeneous acceptor identity, with

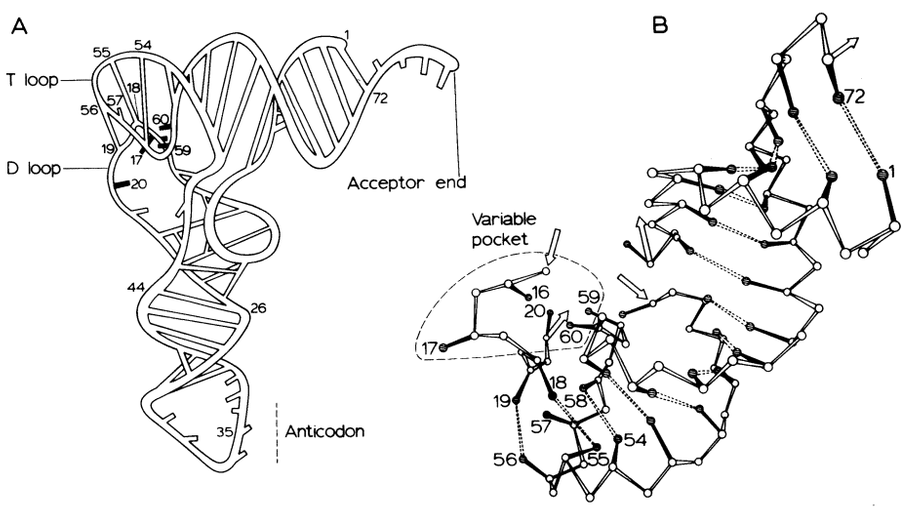

Fig. 4. Models of tertiary structure and variable pocket of yeast $tRNA^{Phe}$ (*8*). (**A**) L-shaped model. Nucleotides 16, 17, 20, 59, and 60 comprising the variable pocket are shaded. (**B**) Detail of the variable pocket. Note that the variable pocket is segregated from constant nucleotides 18, 19, 54, 55, 56, 57, and 58. The acceptor stem and TΨ stem and part of the D (dihydrouridine) loop are shown as viewed by looking down from a point behind and to the right of the 3′ acceptor stem in (A). Bases are represented as hatched circles and hydrogen bonds by dashed lines. Open arrows indicate points where the polynucleotide chain continues to other parts of the molecule.

both Arg and Lys found in suppressed protein (Table 1). Nucleotide C35 in the middle of the anticodon is an identity candidate, as all known isoacceptors of $tRNA^{Arg}$ contain this residue and it appears in all four dinucleotide combinations in the expectation maximization algorithm (Fig. 1). In addition, chemical modification studies have demonstrated that C35 is important for the Arg acceptor activity of $tRNA^{Arg}$ in vitro (*18*).

From our results with suppressor $tRNA^{Arg}$, we conclude that nucleotide A20 in the tRNAs of FTOR1 and PHEA20U20aA59 contributes to the productive interaction with Arg-tRNA synthetase. Further, the minor amounts of amino acid other than Arg found in suppressed protein produced by FTOR1 and PHEA20U20aA59 may reflect the absence of C35 in these tRNAs. It is puzzling that both FTOR1 and PHEA20U20aA59 tRNAs inserted more Arg and less Lys in suppressed protein than did $tRNA^{Arg}$ (75% versus 37% Arg, respectively), but this may reflect different distributions in $tRNA^{Phe}$ and $tRNA^{Arg}$ molecules of nucleotides that repel the Lys-tRNA synthetase. Finally, neither the FTOR1 tRNA nor $tRNA^{Arg}$ has been examined to determine whether nucleotide U20a, which is adjacent to A20, contributes to acceptor identity other than serving as a structural spacer between the constant nucleotides at positions 18 and 21; likewise, positions where $tRNA^{Arg}$ and the FTOR1 tRNA share the same nucleotide (Fig. 2) have not been examined.

An inspection of all available nucleotide sequences of $tRNA^{Arg}$ (*16*) reveals that residue A20 is conserved in life forms except in yeast. This regularity may reflect a conserved feature of the acceptor identity of $tRNA^{Arg}$. The observation that nucleotide 20 contributes to the acceptor identity of *E. coli* $tRNA^{Arg}$ was anticipated from structural and theoretical considerations by Klug and co-workers (*8*). It was noted that the nucleotides at positions 16, 17, 20, 59, and 60 form a patch that arches out from the molecular surface of yeast $tRNA^{Phe}$ (Fig. 4B). The nucleotides in this patch segregate separately from a neighboring cluster of conserved nucleotides; further, there is variation in the number and type of nucleotides within the patch. Thus it was proposed that the patch constitutes a variable pocket that is part of a recognition system used to discriminate different tRNAs, possibly by the aminoacyl-tRNA synthetases. The involvement of the variable pocket in acceptor identity is not limited to $tRNA^{Arg}$, as recent reports have also demonstrated that, in addition to the anticodon (*6, 18*) and acceptor stem (*1, 3, 5, 9, 11–13, 22*), one or more nucleotides in the variable pocket contributes to the acceptor identity of *E. coli* $tRNA^{Ala}$ and $tRNA^{Phe}$ (*9, 11*) and yeast $tRNA^{Phe}$ (*10*).

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Deficit of Spinal Cord Glycine/Strychnine Receptors in Inherited Myoclonus of Poll Hereford Calves

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Inherited myoclonus of Poll Hereford calves is characterized by hyperesthesia and myoclonic jerks of the skeletal musculature, which occur spontaneously and in response to sensory stimuli. The disease shows autosomal recessive inheritance, and significant proportions of the Poll Hereford herds in many countries are thought to be carriers of the mutant gene. Studies revealed a specific and marked (90 to 95 percent) deficit in [^{3}H]strychnine binding sites in spinal cord membranes from myoclonic animals compared to controls, reflecting a loss of, or defect in, glycine/strychnine receptors. Spinal cord synaptosomes prepared from affected animals showed a significantly increased ability to accumulate [^{3}H]glycine, indicating an increased capacity of the high-affinity neuronal uptake system for glycine. In contrast, stimulus-induced glycine release and spinal cord glycine concentrations were unaltered.

INHERITED CONGENITAL MYOCLONUS of Poll Hereford and Poll Hereford cross calves has been reported in the United States, Australia, New Zealand, and England (*1*). This disease, previously (and erroneously) known as neuraxial edema, is characterized by hyperesthesia and myoclonic jerks of the skeletal musculature, which occur spontaneously and in response to tactile, visual, and auditory stimuli (*2*). Since tactile stimuli are sufficient to trigger convulsions, affected animals are unable to stand to feed and, consequently, do not survive. Breeding experiments show that the disease is inherited in an autosomal recessive manner (*3*). No significant pathological lesions are observed in the central nervous system of affected animals (*2*). The clinical symptoms are unaltered by antiepileptic and anticonvulsant drugs at therapeutic doses. However, the symptoms of the disorder suggest a failure of spinal interneuron inhibition and are similar to those in subconvulsive strychnine poisoning. Strychnine blocks the synaptic action of the inhibitory amino acid transmitter glycine (*4*) by interacting with the postsynaptic glycine receptor (*5*). We now report what we believe is the major

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