ly saturated with nitrates during their lifetime (*22*). Thus, solute permeability could be increased by the delayed indirect transfer of gases from soil particles some time after fire (Figs. 1 and 2) or from postfire biogenic production (*18*).

Nitrogen oxides may alter the permeability of the subdermal cuticle, either through direct oxidation effects or after hydration as acids; $HNO_3$ is a strong acid that is capable of increasing the solute permeance of isolated cuticles (*23*), as well as the subdermal cuticle in *Emmenanthe* seeds (*20*), and induces germination at molarities comparable to those generated by smoke (*24*). Additionally, direct dry deposition of both nitric and acetic acids after fire may be important (*25*), but this is likely a short-lived effect as the high ammonium and alkalinity concentrations in ash eventually buffer these acids.

Although smoke-induced germination in *Emmenanthe* is associated with increased solute permeability of the subdermal cuticle, we cannot yet say if this is directly involved in triggering germination, and other roles for $NO_2$ have been proposed (*26*).

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13. Chambers had a center well of 2 ml of sulfanilic acid plus *N*-(1-naphthyl)ethylenediamine dihydrochloride absorbing reagent, and $NO_2$ was assayed spectrophotometrically according to B. E. Saltzman [*Anal. Chem.* **26**, 1949 (1954)].
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17. Soil (to a depth of 3 cm) and litter samples were collected from 315 $cm^2$ under chaparral and homogenized; subsamples were heated at 190°C for 10 min; and smoke, drawn by vacuum through $dH_2O$, was sampled for $NO_2$ spectrophotometrically as in (*13*). Deeper litter layers, higher temperatures, and longer burning durations would generate higher levels than those reported here.
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# Discrete Determinants in Transfer RNA for Editing and Aminoacylation


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During translation errors of aminoacylation are corrected in editing reactions which ensure that an amino acid is stably attached to its corresponding transfer RNA (tRNA). Previous studies have not shown whether the tRNA nucleotides needed for effecting translational editing are the same as or distinct from those required for aminoacylation, but several considerations have suggested that they are the same. Here, designed tRNAs that are highly active for aminoacylation but are not active in translational editing are presented. The editing reaction can be controlled by manipulation of nucleotides at the corner of the L-shaped tRNA. In contrast, these manipulations do not affect aminoacylation. These results demonstrate the segregation of nucleotide determinants for the editing and aminoacylation functions of tRNA.


During aminoacylation of tRNAs for protein synthesis, errors of amino acid activation (by tRNA synthetases) can occur. These errors are corrected by translational editing reactions, some of which require the action of specific tRNAs. Major determinants for aminoacylation of many tRNAs are located in the acceptor stems and anticodons (*1*, *2*). These two regions of the L-shaped tRNA are in different domains that represent the individual arms of the "L." Editing reactions involve the transfer of a misactivated amino acid to the hydroxyl group of a water molecule. This is chemically similar to the transfer of an activated amino acid to the 3′-hydroxyl of a tRNA. Considering the similarity of the reactions and the early demonstration of the close relation between the editing and aminoacylation activity of a tRNA (*3*), nucleotides that are needed for aminoacylation may be sufficient to confer editing activity.

Errors occur as a result of the difficulty that aminoacyl tRNA synthetases have in discriminating between related amino acids. Pairs of amino acids such as valine and isoleucine, which differ by a single methylene group, or threonine and valine, which are isosteric, are difficult to discriminate (*4*). Isoleucyl–tRNA synthetase (IleRS) misactivates valine with a frequency of about 1/180 that of isoleucine activation (*5*). This misactivation is about two orders of magnitude less than the overall


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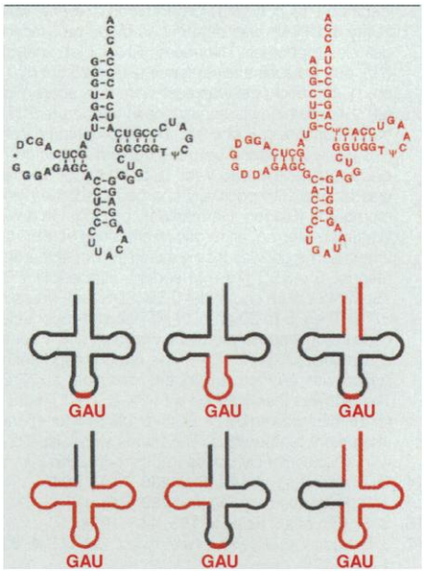


**Fig. 1.** Cloverleaf diagram of $tRNA^{Val}$ (UAC anticodon) and $tRNA^{Ile}$ (GAU anticodon) shown in black and red, respectively, and chimeric molecules that are combinations of specific fragments of $tRNA^{Val}$ and $tRNA^{Ile}$. The common D, Y, and T modified bases in the D- and TYC-stem-loop are shown along with the four major bases. Many nucleotides are the same in the two tRNAs. The chimeric tRNAs are depicted as having $tRNA^{Val}$ as the host (in black) into which a region (for example, GAU anticodon, D-stem-loop) from $tRNA^{Ile}$ was introduced (in red).

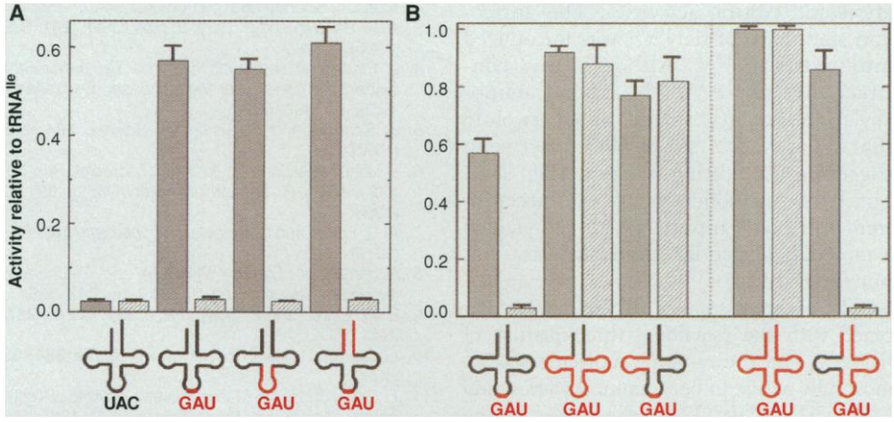


**Fig. 2.** Bar graph comparisons of rates of aminoacylation with isoleucine at pH 7.5, 37°C (gray vertical bars), and of tRNA-dependent hydrolysis of valyladenylate (editing) (white, cross-hatched bars) at pH 7.5, 25°C (*21*). Rates expressed as bars are relative to those of native $tRNA^{Ile}$. Beneath the bar graphs the specific tRNAs (and their anticodon sequences) that were used for the aminoacylation and editing assays are shown; the sequences of these tRNAs are given in Fig. 1. (**A**) Whereas $tRNA^{Val}$ (UAC anticodon) is inactive for aminoacylation with isoleucine, $tRNA^{Val/GAU}$ is active; in spite of its activity for aminoacylation, this molecule does not trigger the editing reaction. Similarly, when either the anticodon stem-loop or the acceptor stem from $tRNA^{Ile}$ is introduced into $tRNA^{Val/GAU}$, the resulting chimeric molecules are highly active for charging but have no activity for triggering hydrolysis of misactivated valyladenylate. (**B**) Whereas $tRNA^{Val/GAU}$ is inactive for triggering the editing response, transplantation (from $tRNA^{Ile}$) of the D-loop together with the anticodon- and TϕC-stem-loop, or of the D-loop alone, yields a molecule that is highly active in the editing reaction. Transplantation of the D-loop from $tRNA^{Val}$ into $tRNA^{Ile}$ had little effect on aminoacylation with isoleucine, but inactivated the $tRNA^{Ile}$ variant for editing. Error bars are standard deviations.

fidelity manifested in protein synthesis (6). Similarly, valyl–tRNA synthetase misactivates threonine at a relatively high frequency compared to the overall accuracy of translation (*7*, *8*).

The overall editing reaction with valine as catalyzed by isoleucyl–tRNA synthetase is

$$\text{IleRS} + \text{Val} + \text{ATP} \rightarrow \text{IleRS(Val-AMP)} + \text{PPi} \quad (1)$$

$$\text{IleRS(Val-AMP)} + \text{tRNA}^{\text{Ile}} \rightarrow \text{IleRS} + \text{Val} + \text{AMP} + \text{tRNA}^{\text{Ile}} \quad (2)$$

In reaction 1, Val is misactivated to form the tightly bound valyladenylate intermediate, and in reaction 2, the addition of isoleucine tRNA ($tRNA^{Ile}$) triggers the hydrolysis of the misactivated adenylate. Some hydrolysis of Val-AMP (adenosine monophosphate) occurs before transfer of the valine to $tRNA^{Ile}$ [pretransfer editing (*8*)], whereas an esterase activity encoded by an insertion (CP1) in the enzyme removes valine from a transiently formed mischarged Val-$tRNA^{Ile}$ species [posttransfer editing (*8*–*10*)]. In either case, the net result is the $tRNA^{Ile}$-dependent hydrolysis of adenosine triphosphate (ATP). (Therefore, editing-induced hydrolysis of ATP measures both pre- and posttransfer editing.) In contrast, when $tRNA^{Ile}$ is added to the correctly activated IleRS(Ile-AMP) complex, there is no breakdown of the isoleucyladenylate, and the isoleucyl moiety is stably joined to $tRNA^{Ile}$. Thus, RNA-dependent amino acid discrimination affords selectivity between isoleucine and valine.

To investigate the structural basis for RNA-dependent amino acid discrimination, we examined whether valine tRNA ($tRNA^{Val}$) could trigger the hydrolysis of misactivated Val-AMP bound to IleRS, under conditions in which the concentration of $tRNA^{Val}$ was high enough to bind to IleRS (*11*). Whereas $tRNA^{Ile}$ was efficient at inducing hydrolysis, we observed no hydrolysis when $tRNA^{Val}$ was added (*12*). These results prompted us to identify the nucleotide differences between $tRNA^{Ile}$ and $tRNA^{Val}$ that were responsible for the editing activity.

We constructed a series of chimeric tRNA molecules (*13*) in which portions of $tRNA^{Val}$ and $tRNA^{Ile}$ were combined (Fig. 1). Because base modification affects the activity of $tRNA^{Ile}$ (*14*), all of the chimeric molecules were produced in vivo and subsequently purified. $tRNA^{Val}$ is not aminoacylated by IleRS, and therefore any change in $tRNA^{Val}$ that would convert it into a substrate for aminoacylation with isoleucine might also make it a viable substrate for editing [reaction (2)]. The anticodon of $tRNA^{Ile}$ is a major determinant for the identity of $tRNA^{Ile}$ (*1*, *14*). When the UAC anticodon of $tRNA^{Val}$ was replaced with the GAU anticodon of $tRNA^{Ile}$, a $tRNA^{Val/GAU}$ species was created that was an efficient substrate for aminoacylation with isoleucyl–tRNA synthetase but not active in triggering the editing response (Fig. 2A). Thus, the nucleotides sufficient for aminoacylation were not able to confer the editing response. Other constructs were made and tested for their aminoacyl-

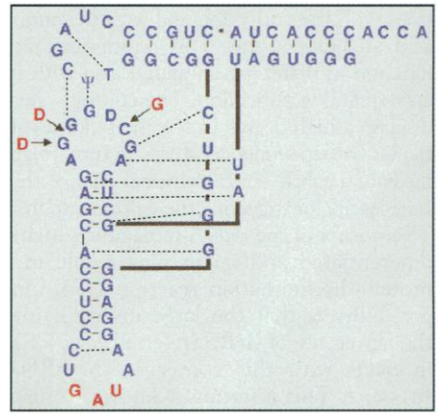

**Fig. 3.** Schematic diagram of $tRNA^{Val/GAU}$ within the L-shaped three-dimensional structure. Dotted lines indicate tertiary interactions. The common modified bases (Y, T, D) in the D- and TYC-loops are shown. The three positions in the D-loop that are needed to trigger the editing response are marked with arrows.

ation and editing activities. The anticodon stem-loop of $tRNA^{Ile}$ was introduced into $tRNA^{Val/GAU}$. Although this construct, like $tRNA^{Val/GAU}$, has an aminoacylation activity within about twofold that of native $tRNA^{Ile}$, it was not active in triggering the editing reaction (Fig. 2A). Similarly, introduction of the acceptor stem of $tRNA^{Ile}$ into $tRNA^{Val/GAU}$ yielded a molecule active for aminoacylation but not for editing.

The acceptor stem of $tRNA^{Val}$ was combined with the remaining three-quarters of $tRNA^{Ile}$, and the resulting molecule was almost fully active in both aminoacylation and editing (Fig. 2B). Thus, the origin of the acceptor stem, whether $tRNA^{Ile}$ or $tRNA^{Val}$, does not affect editing. Instead, the nucleotides required for editing reside in part or all of the D-loop (the sequences of the D-stem of $tRNA^{Ile}$ and $tRNA^{Val}$ are identical) or the TψC-stem-loop of $tRNA^{Ile}$. To test this hypothesis, we installed the D-loop from $tRNA^{Ile}$ into $tRNA^{Val/GAU}$ by changing three nucleotides (including an inserted nucleotide) of $tRNA^{Val/GAU}$. This construct was nearly fully active in aminoacylation and editing (Fig. 2B). Thus, a D-loop swap was sufficient to trigger the editing response but had little or no effect on the aminoacylation function. Next, we introduced the D-loop of $tRNA^{Val}$ into native $tRNA^{Ile}$. This molecule was almost fully active in aminoacylation but was inactive for editing (Fig. 2B). Therefore, regardless of whether the core framework is derived from $tRNA^{Ile}$ or $tRNA^{Val/GAU}$, the sequence of the D-loop is sufficient to trigger the editing response.

The D-loop interacts with the TψC-loop to form the corner of the two-domain L-shaped tRNA molecule (*15–17*) (Fig. 3). In this location the D-loop is positioned to be sensitive to simultaneous synthetase interactions with the anticodon and with the amino acid attachment site. This positioning may function in matching an amino acid with its corresponding anticodon. The editing system displays a high degree of specificity for a particular D-loop sequence which, in turn, might mediate a subtle conformational change that is necessary for triggering the editing response. This corner of the tRNA molecule is highly differentiated and might play a role in a protein-discrimination reaction (*17*). One possibility is that the large insertion into the active site of IleRS (referred to as CP1) interacts with this corner of the tRNA structure. This insertion is known to function in editing (*10*, *18*).

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# Ndj1p, a Meiotic Telomere Protein Required for Normal Chromosome Synapsis and Segregation in Yeast


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The *Saccharomyces cerevisiae* gene *NDJ1* (nondisjunction) encodes a protein that accumulates at telomeres during meiotic prophase. Deletion of *NDJ1* (*ndj1*Δ) caused nondisjunction, impaired distributive segregation of linear chromosomes, and disordered the distribution of telomeric Rap1p, but it did not affect distributive segregation of circular plasmids. Induction of meiotic recombination and the extent of crossing-over were largely normal in *ndj1*Δ cells, but formation of axial elements and synapsis were delayed. Thus, Ndj1p may stabilize homologous DNA interactions at telomeres, and possibly at other sites, and it is required for a telomere activity in distributive segregation.


Efficient segregation of chromosomes in meiosis is required for haploidization. In a screen for yeast genes that control meiotic chromosome segregation, we identified *NDJ1* by its ability to interfere with this process when overexpressed (*1*). *NDJ1* corresponds to the open reading frame *YOL104c* from chromosome XV (*2*). The predicted Ndj1 protein comprises 352 amino acids and shows no significant similarities to other proteins. *NDJ1* was previously identified as the sporulation-specific gene M45 (*3*). A 1.4-kb *NDJ1* mRNA was detected by Northern blot analysis in yeast cells early in meiosis, but it was not detect-


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