except that each ring contained between 14 and 16 particles. Coulton and Murray saw rings of between 14 and 16 particles surrounding annular depressions in membranes at the poles of *Aquaspirillum serpens* (*21*). Taken together, these results suggest that the number of particles in a fully functional *E. coli* motor might be as high as 16. If so, our results would indicate that there are two particles per force generator.

The amino acid sequences of MotA and MotB have been determined from the nucleotide sequences of their genes (*22*). MotB is moderately hydrophobic, and MotA is significantly more so. The MotA sequence contains four strongly hydrophobic segments that are probably transmembrane α helices; the MotB sequence has only one. By using Tn*phoA* insertions into *motB* and controlled proteolysis of MotB, Chun and Parkinson (*23*) have shown that MotB traverses the cytoplasmic membrane once near its NH_2-terminus, with its bulk situated in the periplasm. They suggest that MotB could serve as a linker that connects the torque-generating machinery of the motor to the cell wall. MotA topology has not yet been examined as closely, but its four hydrophobic segments together contain enough moderately polar, hydrogen-bonding residues (for example, Ser, Thr, Asn, and His) to enable them to form a proton-conducting channel. If so, each pair of membrane particles, and each force generator, might comprise one or more MotB linkers and MotA channels.

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Association of Transfer RNA Acceptor Identity with a Helical Irregularity

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The aminoacylation specificity ("acceptor identity") of transfer RNAs (tRNAs) has previously been associated with the position of particular nucleotides, as opposed to distinctive elements of three-dimensional structure. The contribution of a G·U wobble pair in the acceptor helix of $tRNA^{Ala}$ to acceptor identity was examined with synthetic amber suppressor tRNAs in *Escherichia coli*. The acceptor identity was not affected by replacing the G·U wobble pair in $tRNA^{Ala}$ with a G·A, C·A, or U·U wobble pair. Furthermore, a $tRNA^{Ala}$ acceptor identity was conferred on $tRNA^{Lys}$ when the same site in the acceptor helix was replaced with any of several wobble pairs. Additional data with $tRNA^{Ala}$ show that a substantial acceptor identity was retained when the G·U wobble pair was translocated to another site in the acceptor helix. These results suggest that the G·U wobble pair induces an irregularity in the acceptor helix of $tRNA^{Ala}$ to match a complementary structure in the aminoacylating enzyme.

THE ACCEPTOR IDENTITY OF *ESCHERICHIA COLI* $tRNA^{Ala}$ is partially determined by a G·U wobble pair in the acceptor stem of the molecule. The acceptor stem is helical, and this structural feature is generally assumed to be common to all tRNAs. The importance of this G·U wobble pair to $tRNA^{Ala}$ acceptor identity was initially suggested in biochemical and genetic experiments (*1*), and was eventually established by replacing the wild-type base pair in other tRNAs with a G·U wobble pair and observing acceptor identity changing to $tRNA^{Ala}$ (*2, 3*). The wobble pairing between G and U displaces these bases relative to those of Watson-Crick pairs, giving the grooves of the helix a distinctive pattern of functional groups (*4*). The available data indicate that the G·U wobble pair is incor-

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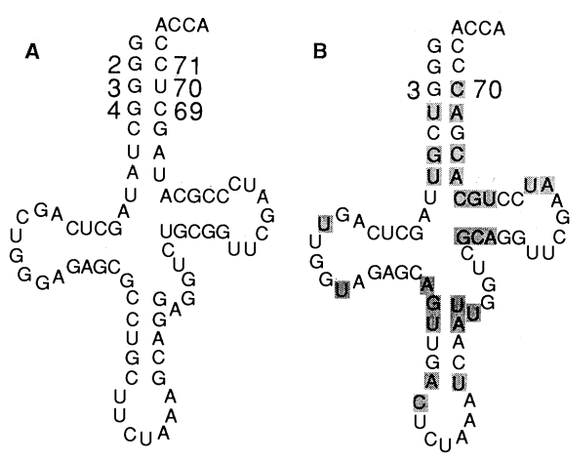

Fig. 1. Nucleotide sequence of the amber suppressors of (**A**) $tRNA^{Ala}$ (*2*) and (**B**) $tRNA^{Lys}$ (*8*). Numbers identify base pair positions in the acceptor helix. Shaded nucleotides in $tRNA^{Lys}$ differ from those in $tRNA^{Ala}$.

porated into the helix without marked changes in global conformation (*5*), although the helix is destabilized (*6*). The distinctive pattern of functional groups on G and U nucleotides could lead to a specific interaction between $tRNA^{Ala}$ and its cognate aminoacylating enzyme by using complementary functional groups on interacting molecules. This interaction would also provide a discrimination factor against other tRNAs because they do not contain this structural feature. Alternatively, a different structural aspect of the G·U wobble pair in $tRNA^{Ala}$ could contribute to the interaction with the cognate enzyme.

We reasoned that replacing the G·U wobble pair in $tRNA^{Ala}$ with other base combinations would change acceptor identity if the distinctive pattern of functional groups on the G and U nucleotides are necessary for acceptor identity. Alternatively, acceptor identity would not necessarily change if another aspect of the structure contributes. Sixteen synthetic genes containing the nucleotide sequence of amber suppressor $tRNA^{Ala}$ (*2*) (Fig. 1) were prepared and cloned in *E. coli* for a functional analysis. These gene sequences of $tRNA^{Ala}$ differed only in the base combination present at the site of the G·U wobble pair ("position 3·70") in the acceptor helix. For the functional analysis (*7*), acceptor identity was initially characterized by the ability to suppress the auxotrophy associated with amber mutations in the *TrpA* gene of *E. coli*; acceptor identity was then directly established by sequencing a suppressed protein (dihydrofolate reductase) to determine the amino acid inserted by the suppressor tRNA (*2, 8, 9*). Three inefficient suppressor tRNAs containing G·G, A·A, or A·G were not completely characterized. Comprehensive analyses of the remaining variants revealed that each had at least a partial $tRNA^{Ala}$ acceptor identity (Table 1). Remarkably, the acceptor identity of variants with G·A, C·A, and U·U at position 3·70 in $tRNA^{Ala}$ was not distinguished from wild-type $tRNA^{Ala}$ with G·U at this position. Structural studies with DNA fragments (*5*) suggest that G·A, C·A, U·U, and C·C can form wobble pairs in $tRNA^{Ala}$ that are somewhat similar to the G·U wobble pair. Other variants with A·C and C·C at position 3·70 in $tRNA^{Ala}$ had acceptor identities that were substantially that of $tRNA^{Ala}$, but that also had some $tRNA^{Gln}$ identity. The $tRNA^{Gln}$ acceptor identity of the latter variants may reflect the presence of a positive signal for $tRNA^{Gln}$ acceptor identity rather than a negative signal for $tRNA^{Ala}$ acceptor identity, since all four variants with C at position 70 had a partial $tRNA^{Gln}$ acceptor identity.

These results establish that the G·U wobble pair at position 3·70 in $tRNA^{Ala}$ is not necessary for the specificity of acceptor identity. Furthermore, a comparison of the base combinations that are functionally similar to G·U (that is, G·A, C·A, and U·U, and possibly A·C and C·C) shows no logical pattern of functional groups at equivalent base positions that might serve to discriminate them and G·U from all others. Thus a different aspect of structure contributes to acceptor identity. The G·U wobble pair could induce an irregular structure in the $tRNA^{Ala}$ acceptor helix to match a complementary structure in the Ala-tRNA synthetase. As the three-dimensional structure of $tRNA^{Ala}$ has not been determined, its acceptor stem may not have the A-type helix observed in other tRNA structures, including those of yeast $tRNA^{Phe}$ and $tRNA^{Asp}$, which also contain a G·U wobble pair in the acceptor helix (*5*). An irregularity arising from the G·U wobble pair in $tRNA^{Ala}$ could exist either as a static or dynamic aspect of the structure. The variant tRNAs with Watson-Crick pairs at this site form this structure with more difficulty or use other regions of the molecule (*2*) to achieve the partial $tRNA^{Ala}$ acceptor identity which

Table 1. Functional properties of suppressor tRNAs. Shown are the base combinations at position 3·70 in simple variants of $tRNA^{Ala}$ and $tRNA^{Lys}$. The base combinations at three positions (2·71, 3·70, and 4·69, respectively) are shown in complex variants of $tRNA^{Ala}$. Bold facing indicates mutant bases. Genes for suppressor tRNAs were made from synthetic oligonucleotides. Suppressed dihydrofolate reductase protein was purified and sequenced through residue 15 and amino acid percentages ≥5% at residue 10, corresponding to the amber codon, are reported. Values for Gln include Glu (Glu was ≥20% of the total). Cys was not analyzed. The data indicated that the proteins were homogeneous, except from $tRNA^{Ala}$ U3·A70, which was judged to be 70% pure based on amino acid yields at residues other than 10 (this value has not been corrected for purity). The small amount of protein analyzed for $tRNA^{Ala}$ C3·U70 precluded a reliable determination. Average values based on determinations from two independent preparations (individual values were ±3% of respective average value) are reported for protein from $tRNA^{Lys}$ G3·U70, $tRNA^{Lys}$ A3·U70, $tRNA^{Ala}$ G2·C71, A3·U70, G4·U69, and $tRNA^{Ala}$ C2·G71, G3·U70, C4·G69. The suppression of auxotrophy in *TrpA* was scored as follows: +, growth; +/− and −/+, partial growth (+/− is more growth than −/+); and −, no growth. Amino acids that yielded functional *TrpA* polypeptides for these alleles are: 15 and 243, many; 49, acidic; 211, Ala, Gly, Cys, Ser, Thr, Ile, Leu, Val, and Asn; and 234, Ala and Gly. The suppression efficiency is reported for amber allele A16 in the *lacI-Z* system. The value is the percentage of the wild-type *lacI-Z*40 fusion, which averaged 215 units. The value for cells without a suppressor gene was <0.001%. The methods have been described previously (*2, 8, 9*).

Suppressor gene	Amino acid in suppressed protein (%)			Suppression of auxotrophy at *TrpA* allele*			Suppression efficiency (%)
	Ala	Lys	Gln	15	211	243	
			$tRNA^{Ala}$ (position 3 · 70)				
G · U	96			+/−	+	+/−	4.12
G · C	18	29	44	+/−	−	+/−	0.07
G · A	89			+/−	+/−	+/−	0.63
G · G†				−	−	−	0.01
A · U	55	29	6	+/−	+/−	+/−	0.39
A · C	83		12	+/−	+/−	+/−	0.55
A · A†				−	−	−	0.00
A · G‡				−/+	−	−/+	0.02
C · U	~90			+/−	+/−	+/−	0.03
C · C	78		13	+/−	−/+	+/−	0.44
C · A	91			+	+/−	+	0.55
C · G	53	10	26	−	−	−	0.14
U · U	90			+	+/−	+	0.36
U · C	13	6	74	+/−	−	+/−	0.06
U · A	~58	~12		+/−	+/−	+/−	0.68
U · G	75	12		−/+	−	−/+	0.22
			$tRNA^{Lys}$ (position 3 · 70)				
G · U	94			+/−	+/−	+/−	34.30
G · C		94		+	−	+	30.88
G · A	39	49		+	+	+	1.61
A · U		95		+	−/+	+	4.82
A · C	22	69		+	+/−	+	3.63
U · U		89§		+	+/−	+	8.37
U · C		85	6	+	−	+	7.58
			$tRNA^{Ala}$ (positions 2 · 71, 3 · 70, and 4 · 69)				
G · C, **A · U**, G · **U**	79	7		+/−	+/−	+/−	0.94
G · C, G · **C**, G · **U**‡				+/−	−	+/−	0.00
G · **U**, **A** · U, G · C	19	23	32‖	+/−	−	+/−	0.04
C · G, G · U, **C · G**	85¶			+/−	+/−	+/−	1.37

*For allele 49, all variants were scored −; for allele 234, all variants were scored − except $tRNA^{Lys}$ G · U (+/−) and G · A and A · C (−/+). †Protein not analyzed. ‡Insufficient protein for sequencing. §Also Trp, 5%. ‖Also Gly, 8%, and Met, 6%. ¶Also Arg, 6%.

they display. The existence of other regions in $tRNA^{Ala}$ that contribute to acceptor identity, noted previously (*2*), is further demonstrated by the retention of a partial $tRNA^{Ala}$ acceptor identity even in the most extreme variants with G·C or U·C at position 3·70 (Table 1).

The 16 suppressors of $tRNA^{Ala}$ on an agar plate containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside are shown in Fig. 2. On these indicator plates, cells with active suppressor tRNAs form blue colonies and cells with less active or inactive suppressor tRNAs form light blue or white colonies, respectively. Several color groups are distinguished: blue (G·U), light blue (G·A, A·C, C·C, C·A, U·U, A·U, and U·A) and white (G·C, G·G, A·A, A·G, C·U, C·G, U·C, and U·G). The light blue and white groups are suggestive of weaker suppressor tRNAs. The results of quantitative measurements in liquid cultures confirm that every change away from G·U is accompanied by a reduction in the efficiency of suppression (Table 1). The cellular level of mature suppressor tRNA was not reduced, except in the variants with C·U and C·C (*10*). Thus the depression in the efficiency of suppression observed with all variants means that suppressor $tRNA^{Ala}$ is functionally damaged. This qualitative alteration may be expressed at the level of tRNA aminoacylation or during the ribosomal performance of the tRNA. Damage to the aminoacylation of the tRNA would suggest that a functional group on the G·U wobble pair contacts the aminoacylating enzyme, or that the G·U wobble pair is superior to all other base combinations in directing the formation of the structural irregularity. Different factors, such as steric interference (for example, G at position 70), may also be involved. Other methods (*11*) must be used to address the complexity of this system.

It should be possible to change the acceptor identity of another tRNA to that of $tRNA^{Ala}$ with any one of the several different wobble pairs described above if each of these contributes to the productive interaction between tRNA and the Ala-tRNA synthetase. An amber suppressor gene of $tRNA^{Lys}$ (*8*) with a G·U wobble pair replacing the wild-type G·C base pair at position 3·70 had a $tRNA^{Ala}$ acceptor identity, whereas the variants with either a G·A or A·C wobble pair had a partial $tRNA^{Ala}$ acceptor identity (Table 1). These results confirm the contribution of the nucleotides at position 3·70 to $tRNA^{Ala}$ acceptor identity and emphasize the functional competence of several different wobble pairs at this position. Although replacing the G·C base pair with an A·U base pair at position 3·70 in $tRNA^{Lys}$ did not affect the acceptor identity (Table 1) or the cellular level of the suppressor tRNA (*10*), it did reduce the suppression efficiency (Table 1). If the G·C at position 3·70 in $tRNA^{Lys}$ contributes to $tRNA^{Lys}$ acceptor identity, then acceptor identity transformations from $tRNA^{Lys}$ to $tRNA^{Ala}$ may be facilitated.

We have suggested that the G·U wobble pair influences $tRNA^{Ala}$ acceptor identity by inducing an irregularity in the acceptor helix of the molecule. Depending on the nature and extent of this irregularity, it might be possible to translocate the G·U wobble pair to another site in the acceptor helix and retain acceptor identity. To test this idea it was necessary to change position 3·70 in $tRNA^{Ala}$ from G·U to a Watson-Crick pair to damage the normal acceptor identity. The results of these experiments (Table 1 and Fig. 2) show that $tRNA^{Ala}$ with a G·U wobble pair at position 4·69 and an A·U base pair at position 3·70 was stimulated for both $tRNA^{Ala}$ acceptor identity and suppression efficiency compared with $tRNA^{Ala}$ that only had the A·U base pair at position 3·70. However, when the G·U wobble pair at position 4·69 was combined with a G·C base pair at position 3·70, the resulting suppressor tRNA was inactive (Table 1) even though the cellular level of the suppressor tRNA was not reduced (*10*). The extra hydrogen bond of the G·C versus the A·U base pair at position 3·70 may be more difficult for the G·U wobble pair at position 4·69 to distort. We cannot account for the enhanced $tRNA^{Ala}$ acceptor identity observed when G·U at position 4·69 is combined with A·U at position 3·70 simply by assuming an indirect stimulation resulting from partial damage to the $tRNA^{Lys}$ and $tRNA^{Gln}$ components of the acceptor identity. The latter assumption would predict that when G·U at 4·69 is combined with G·C at position 3·70 (which is the wild-type base pair in $tRNA^{Lys}$ and $tRNA^{Gln}$), the resulting $tRNA^{Ala}$ would be more active for $tRNA^{Lys}$ and $tRNA^{Gln}$ acceptor identities than $tRNA^{Ala}$ with G·U at position 4·69 and A·U at position 3·70. We found just the opposite.

When we replaced the Watson-Crick pair at position 2·71 with a G·U wobble pair in

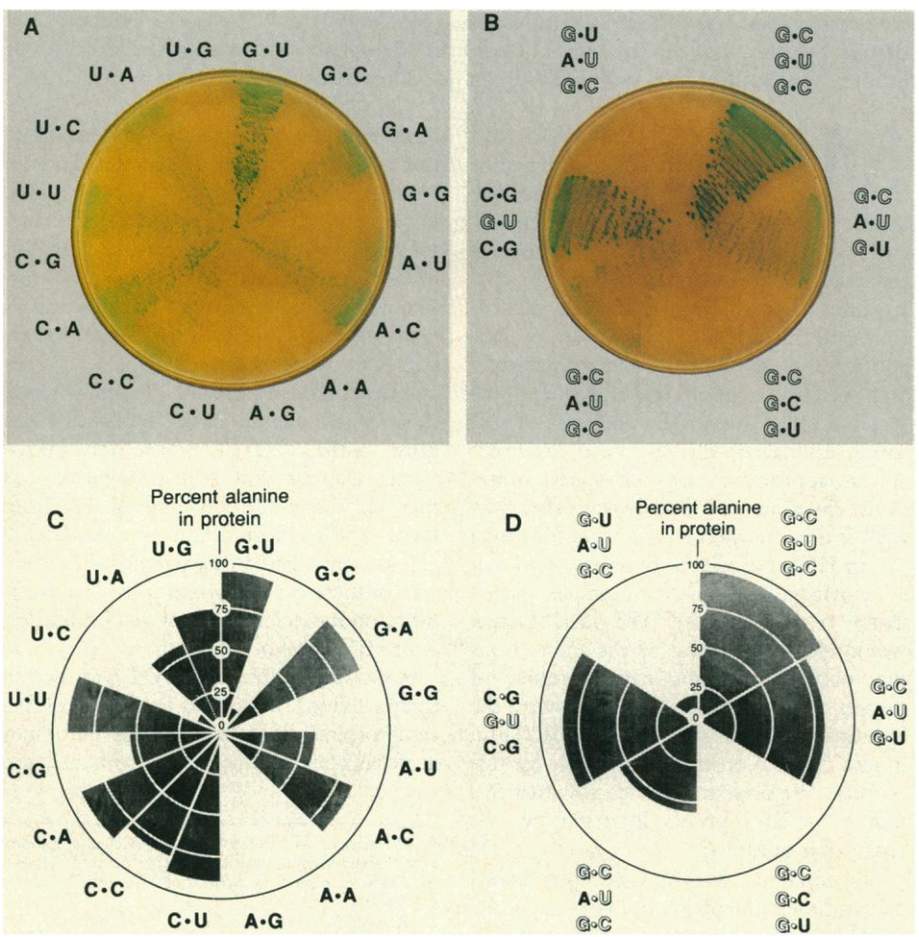

Fig. 2. Activity and acceptor identity of $tRNA^{Ala}$ suppressors. (**A** and **B**) Streak plate containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Cells with active or inactive $tRNA^{Ala}$ suppressors form blue or white colonies, respectively (white colonies photographed poorly). (**C** and **D**) Histogram of the percentage of Ala in the suppressed dihydrofolate reductase protein (from Table 1).

combination with an A·U base pair at position 3·70, the resulting tRNA was severely damaged for acceptor identity and efficiency of suppression (Table 1), although the amount of tRNA was not reduced (*10*). Interpretation of this result is complicated because in this variant the G·U wobble pair is located nearer the 3′ end of the tRNA molecule, and also because this G·U is surrounded by a distinctive base pair context relative to the G·U in other active variants. We have shown that adjacent base pairs can degrade the function of the G·U wobble pair in wild-type $tRNA^{Ala}$. When wild-type $tRNA^{Ala}$ was changed at position 2·71 and 4·69 from G·C to C·G, both the acceptor identity and the suppression efficiency were depressed (variant C2·G71, G3·U70, C4·G69, Table 1).

The contribution of the G·U wobble pair to the acceptor identity of $tRNA^{Ala}$ has been known for some time. We have suggested that the G·U wobble pair induces an irregularity in the acceptor helix that allows $tRNA^{Ala}$ to fit with the cognate synthetase. Other tRNAs lack this structure and are excluded. The structure could kink the helix, or swing nucleotides outward from the helix, or dissociate the helix to two single strands. In all cases, the thermodynamic instability observed with G·U wobble pairs in helices (*6*) may help form the irregularity. The structure could be important in the initial interaction between $tRNA^{Ala}$ and its cognate synthetase, or in a subsequent kinetic step of the reaction. There is increasing evidence from a number of systems that sequence-dependent conformations of duplex nucleic acids can influence the functions of these molecules (*12*). Furthermore, a possible relation between an imperfection in the acceptor helix and $tRNA^{Gln}$ acceptor identity has been noted previously (*13*).

We are only beginning to understand the relation between structure and function in $tRNA^{Ala}$. We have emphasized the importance of an irregular structure to the acceptor identity of $tRNA^{Ala}$, but have noted that a functional group on the G·U wobble pair may also contribute. In addition, other regions of the $tRNA^{Ala}$ molecule are known to be important for acceptor identity (*2*).

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Infection of the SCID-hu Mouse by HIV-1

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SCID-hu mice with human fetal thymic or lymph node implants were inoculated with the cloned human immunodeficiency virus–1 isolate, HIV-1_{JR-CSF}. In a time- and dose-dependent fashion, viral replication spread within the human lymphoid organs. Combination immunohistochemistry and in situ hybridization revealed only viral RNA transcripts in most infected cells, but some cells had both detectable viral transcripts and viral protein. Infected cells were always more apparent in the medulla than in the cortex of the thymus. These studies demonstrate that an acute infection of human lymphoid organs with HIV-1 can be followed in the SCID-hu mouse.

MULTIPLE LINES OF EVIDENCE suggest that the etiologic agent of the acquired immunodeficiency syndrome (AIDS) is human immunodeficiency virus (HIV) (*1*). Definitive proof and direct analysis of infection by HIV in vivo has been hampered by the lack of a suitable small animal model. Thus, we know little about the events which occur immediately after infection or about the subsequent progression of disease (*2*). A number of experimental animal models have been advanced. All are approximations with severe limitations: either an animal retrovirus other than HIV is used to infect a host other than man (*3*), or HIV is used in a context beyond that of its natural tropism (for example, in primates or rabbits) (*4*). The ideal system would allow the study of the interactions that occur during the natural course of disease, that is, those of HIV with the human lymphoid system. The SCID-hu mouse (*5*) was constructed precisely for this purpose. We present evidence now that this mouse model supports infection by and replication of HIV-1.

Implantation, growth, and interactions between the human lymphoid organs of the SCID-hu mouse have been described (*5*). In many respects, the environment of this hematochimeric mouse is similar to that encountered by HIV in man. After parenteral inoculation or sexual contact, it seems likely that HIV drains to and is sequestered within lymphoid organs such as lymph nodes, Peyer's patches, or spleen; during perinatal infection, the developing thymus may also be a major target organ for infection (*6*). These lymphoid organs present full complements of interactive cells which are permissive for infection, including $CD4^+$ T cells and myelomonocytic cells. The human fetal thymus and lymph node implants which grow in the SCID-hu mouse have microscopic features and cellular compositions that are essentially indistinguishable from those of their normal human counterparts (*5*). We anticipated that parenteral inoculation of these organs would provide a previously unobtainable view of acute infection with HIV in vivo.

A molecular clone of HIV-1 was used in these studies (*7*). Derived from the cell-free cerebrospinal fluid of a patient with subacute encephalopathy and Kaposi's sarcoma,

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