A Mechanosensitive Ion Channel in the Yeast Plasma Membrane

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Mechanosensitive ion channels use mechanical energy to gate the dissipation of electrochemical gradients across cell membranes. This function is fundamental to physiological processes such as hearing and touch. In electrophysiological studies of ion channels in the plasma membrane of the yeast *Saccharomyces cerevisiae*, channels were observed that were activated by, and adapted to, stretching of the membrane. Adaptation of channel activity to mechanical stimuli was voltage-dependent. Because these mechanosensitive channels pass both cations and anions, they may play a role in turgor regulation in this walled organism.

A COMMON CELLULAR RESPONSE TO changes in extracellular osmolarity or to direct mechanical deformation of the cell is a change in the ion permeability of the plasma membrane (*1*). The recent demonstration of mechanosensitive ion channels (MS channels) in animal cells (*2*) has suggested a molecular mechanism for membrane stress–elicited permeability changes. We now report the discovery and characterization of an MS channel in the plasma membrane of yeast (*Saccharomyces cerevisiae*) spheroplasts.

After formation of a gigaohm seal (*3*) against the plasma membrane of a spheroplast, quantum fluctuations of picoampere currents were observed on application of negative or positive pressure to the cell surface through the pipette. Pressure-activated currents were also observed in excised inside-out patches (Fig. 1) and in whole-cell recordings. Leakage currents remained the same before and after pressure application. Increasing positive pressure to the patch increased the frequency of current fluctuations from the leakage current level but not the magnitude of the smallest current step (unitary current). We refer to the pressure-activated currents as passing through MS channels. These channels differed from K^+ channels of the same membrane (*4*) in inhibitor sensitivity, selectivity, and conductance (Fig. 2). Gadolinium (10 μM, internal) and tetraethylammonium (30 mM, external) were specific inhibitors for the MS and K^+ channels, respectively (*4–6*).

To examine the ionic selectivity of MS channels, we measured the unitary current-voltage relation of MS channels activated by low pressure in the whole-cell mode (*7*) under bi-ionic conditions, CsCl inside the cell (with Cs^+ replacing K^+ in the cell, K^+ channel currents were not observed) and equal concentrations of the chloride salts of other alkali cations outside the cell (Fig. 2A). The conductance of the smallest open state of MS channels in symmetrical CsCl was 36 pS. When other alkali cations were substituted for Cs^+ outside, only small changes in the reversal potential (E_{rev}) and slope conductance (Γ) were observed (Table 1). These results suggest that MS channels discriminated poorly among alkali cations.

To test for discrimination between cations and anions, the MS channel current-voltage relation was examined in symmetrical solutions containing 170 mM CsCl and then again after equimolal substitution of sorbitol for 80% of the external CsCl (Fig. 2B). The E_{rev} of 0 mV in symmetrical CsCl solutions shifted to -7 ± 2 mV ($n = 4$) after we decreased the external CsCl concentration. The MS channel conductance appeared slightly more selective than that of the leakage conductance for which the E_{rev} remained approximately 0 mV. When E_{rev} was used to calculate ion permeability ratios (legend to Table 1), P_{Cs}/P_{Cl} was 0.6. To further assess whether both cations and anions readily pass through MS channels, we replaced all external CsCl with sorbitol by extensive perfusion. Clear outward (carried by Cs^+) and inward (Cl^-) MS channel currents were observed (Fig. 2C). The selectivity of MS channels for a variety of cations and anions was determined by use of E_{rev} measurements under bi-ionic conditions. These measurements were obtained in whole-cell recordings from either unitary current-voltage plots (Fig. 2A) or by voltage ramps in the presence of applied pressure (Fig. 2B). The E_{rev}'s measured with voltage ramps were independent of the applied pressure. Both methods of determining E_{rev} gave nearly identical results (Table 1). The permeability series for different ion classes were the following: monovalent cations, $K^+ \geq Cs^+ \geq Rb^+ > Na^+ > Li^+ >> Arg^+$; divalent cations: $Cs^+ > Ba^{2+} > Ca^{2+} > Mg^{2+} > Co^{2+}$; and monovalent anions, $I^- > Br^- > Cl^- > F^- >> Glu^-$. Hence, MS channels were not equally open to all ions and were relatively impermeant to Arg^+ and Glu^-.

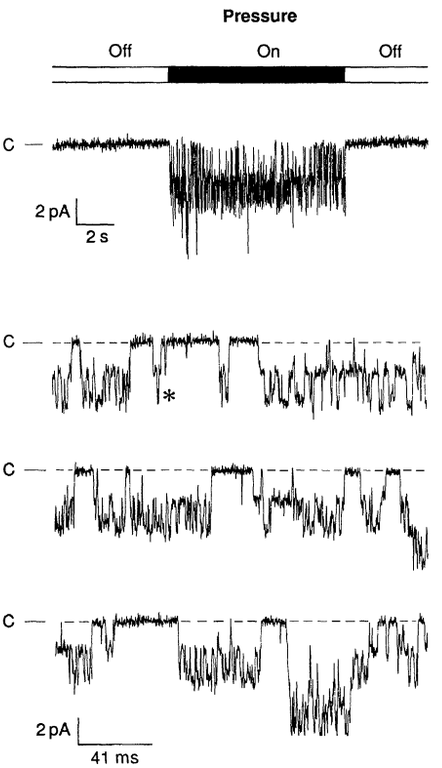

Fig. 1. MS channel activity. On applying 29 kN m^{-2} negative pressure to the external surface of a patch held at -60 mV, fluctuations of inward current were observed (top trace). Viewed at higher time resolution (bottom three traces), the current fluctuations appeared quantal. Typically, the conductance changed in a two-step manner (*), with both steps approximately equal in size. Although most patches contained pressure-dependent conductances, a few patches did not. Procedures for preparing and patch clamping spheroplasts of diploid *S. cerevisiae* were as described (*3, 4*). After gigaohm seal formation, patch excision was achieved by an abrupt change in perfusion velocity. Solutions were 170 mM CsCl, 5 mM $MgCl_2$, 0.1 mM EGTA, and 5 mM Cs^+ Hepes, pH 7.2 (pipette) and 20 mM KCl, 100 mM NaCl, 50 mM $MgCl_2$, 0.1 mM EGTA, and 5 mM K^+ Hepes, pH 7.2 (bath). Temperature for all experiments was 19° to 22°C. Pressure was applied pneumatically to the back of the pipette through a port on the pipette holder and monitored with an in-line pressure transducer (differential-type, ±5 psi, Omega Engineering) calibrated with a mercury manometer. A 5-ml syringe, together with two in-line, three-way valves, was used to apply step changes in pressure. We recorded channel currents on video tape and filtered them at 2 kHz (8-pole Bessel filter) before digitizing (at 10 kHz) and analyzing on an IN-DEC Systems 11/23 computer. C indicates the closed channel current level.

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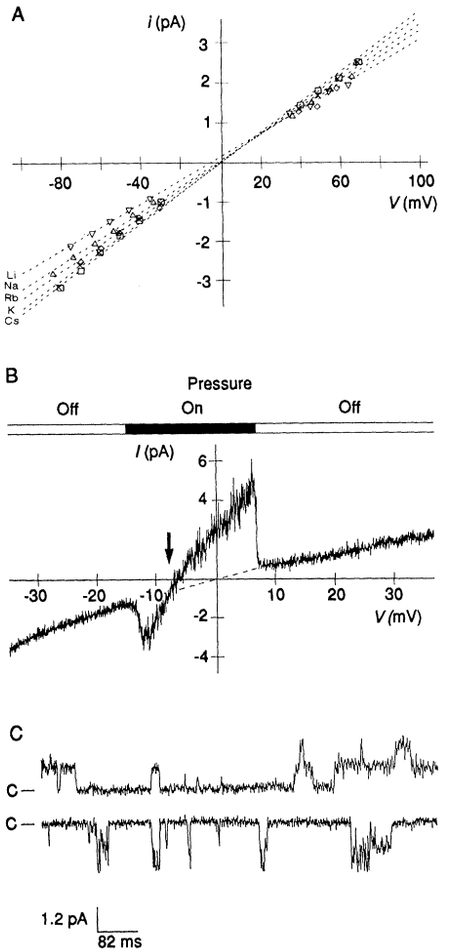

Fig. 2. Ion selectivity of MS channels. (**A**) Unitary current-voltage relation in the whole cell. Currents through MS channels (*21*) were elicited by small, suprathreshold pressures at different voltages. The pipette solution was 170 m*M* CsCl, 5 m*M* $MgCl_2$, 0.1 m*M* EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2. The bath solution was either the same as the pipette solution (■, Cs^+) or the same but with Cs^+ replaced with an equal concentration of K^+ (+), Rb^+ (◇), Na^+ (△), or Li^+ (▽). Liquid junction potentials at the agar-bridge bath electrode were determined separately with a pipette filled with 3*M* KCl. All voltages shown were corrected for these offset voltages, which were <6 mV. Small offset voltages were occasionally observed in symmetrical Cs^+ solutions and these were subtracted from all voltages. (**B**) Macroscopic MS channel current-voltage relation in the whole cell. To obtain the E_{rev}'s for the leakage current and MS channel current (arrow), voltage was "ramped" between −60 mV to +60 mV over 60 s. At the position marked with an arrow, approximately 5 kN m^{-2} positive pressure was applied. Measurements were taken in 170 m*M* CsCl, 5 m*M* $MgCl_2$, 0.1 m*M* EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2 (pipette solution) and 30 m*M* CsCl, 5 m*M* $MgCl_2$, 280 m*M* sorbitol, 0.1 m*M* EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2 (bath solution). (**C**) Cs^+ (top) and Cl^- (bottom) currents through MS channels in a whole-cell recording. The voltages were ±60 mV. Solutions were 170 m*M* CsCl, 5 m*M* $MgCl_2$, 0.1 m*M* EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2 (pipette) and 360 m*M* sorbitol (bath). The series resistance was 0.6 gigaohm and the leakage resistance, 30 gigaohms. C is as defined in Fig. 1. Data are from single representative cells.

We next directly assessed whether pressure (stress normal to the plane of the membrane) or tension (stress in the plane of the membrane) produced the strain energy that regulates MS-channel activity. According to Laplace's law, for a thin-walled sphere at equilibrium, $T = Pd/4$, where T = tension on the membrane, P = applied pressure, and d = cell diameter. Therefore, for channels regulated by membrane tension, open probability will be a function of both the applied pressure and the cell size. For channels regulated by pressure, MS channel open probability will be cell-size independent. The pressure-dependence of MS channel open probability was inversely dependent on the cell diameter (Fig. 3A). When MS-channel currents were plotted as a func-

Table 1. Ion selectivity of MS channels in whole-cell recordings. E_{rev}'s were used to calculate P_{Cs}/P_X (where X = other ions) with the Goldman-Hodgkin-Katz equation modified for the presence of divalent ions (*20*). With bi-ionic solutions (Cs in the pipette and X in the bath), E_{rev}'s were obtained by two different methods, both of which showed good agreement. One method was to determine E_{rev} by interpolation of single MS channel current-voltage plots (Fig. 2A). A second method was to use voltage ramps during pressure application (Fig. 2B) and to read E_{rev} from the intersection of the channel currents with the extrapolated leakage current. Solutions were 170 m*M* CsCl, 5 m*M* $MgCl_2$, 0.1 m*M* Cs^+ EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2 (pipette) and 170 m*M* XCl, 5 m*M* $MgCl_2$, 0.1 m*M* X^+ EGTA, and 5 m*M* X^+ Hepes, *p*H 7.2 (X = monovalent cation, bath) or 120 m*M* YCl_2, and 5 m*M* Y^{2+} Hepes, *p*H 7.2 (Y = divalent cation, bath) or 170 m*M* CsZ, 5 m*M* $MgCl_2$, and 5 Cs^+ Hepes, *p*H 7.2 (Z = monovalent anion, bath). P_{Cs}/P_{Cl} was determined by E_{rev}'s from salt gradient experiments (Fig. 2B). P_{Cs}/P_Z was calculated as $(P_{Cs}/P_{Cl})\cdot(P_{Cl}/P_Z)$. All voltages were corrected for liquid junction potential (Fig. 2, legend). The Γ were taken from single channel current/voltage (i/V) plots. E_{rev}'s, Γ are means ± SD; P_X/P_{Cs} are means ± SE; n = number of cells used. Data from two measurements are averages; * indicates where i/V was used to measure E_{rev}.

Ion	E_{rev} (mV)	n	Γ (pS)	P_X/P_{Cs}
Cs^+	+0.8 ± 2.0	14	36 ± 2	1.06 ± 0.08*
K^+	+1.3 ± 1.1	4	36 ± 2	1.09 ± 0.04*
Rb^+	−1.3 ± 2.4	3	36 ± 3	0.91 ± 0.09*
Na^+	−3.9 ± 1.6	3	32 ± 1	0.75 ± 0.05*
Li^+	−10.4 ± 3.8	3	30 ± 2	0.42 ± 0.10*
Ba^{2+}	−2.1 ± 1.8	4	35 ± 1	0.76 ± 0.06*
Ca^{2+}	−5.9 ± 2.6	4	32 ± 2	0.54 ± 0.06*
Mg^{2+}	−7.7 ± 1.3	5	32 ± 2	0.46 ± 0.02*
Co^{2+}	−11	2	28	0.32*
Arg^+	−20	2		0.05
Cl^-	0	2		0.63
F^-	+1	2		0.57
Br^-	−6	2		1.09
I^-	−9	2		1.37
Glu^-	+10	2		0.07

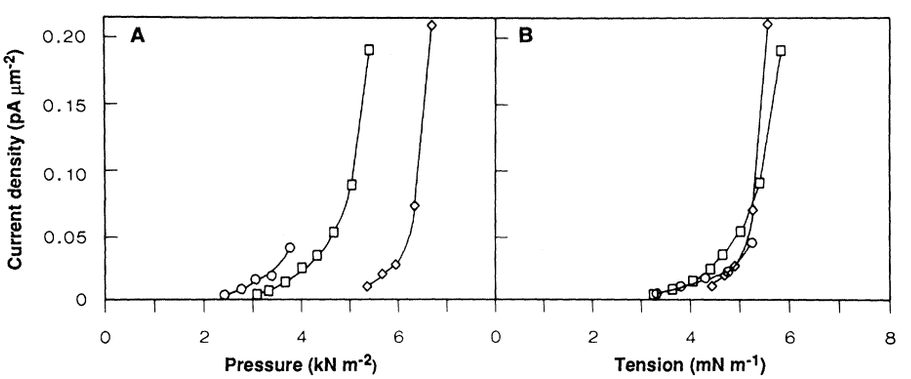

Fig. 3. Current density, through MS channels of three cells of different sizes, plotted against the applied pressure or tension. (**A**) Pressure-dependence of MS channels in three whole-cell recordings. Cell diameters were 5.7 μm (○), 4.3 μm (□), and 3.3 μm (◇). Pressure was applied in steps of 30-s duration. At low pressure, repetitive steps of the same pressure yielded the same mean current through MS channels (*5*). Adaptation was not noted (Fig. 4). Pressure was measured as in Fig. 1. (**B**) Tension dependence of MS channels in the same three cells. Tension was calculated from Laplace's law and is the mean or isotropic tension for the cell membrane. The voltage was −60 mV. Surface area and diameter of each spheroplast were estimated from capacitance measurements after achieving the whole-cell recording mode. A membrane capacitance of 1 pF per 100 μm^2 (*22*) was assumed. Solutions were 170 m*M* CsCl, 5 m*M* $MgCl_2$, 0.1 m*M* EGTA, and 5 m*M* Cs^+ Hepes, *p*H 7.2 (pipette and bath). These three cells were from a single batch of spheroplasts; the results were representative of those observed with at least three other groups of cells.

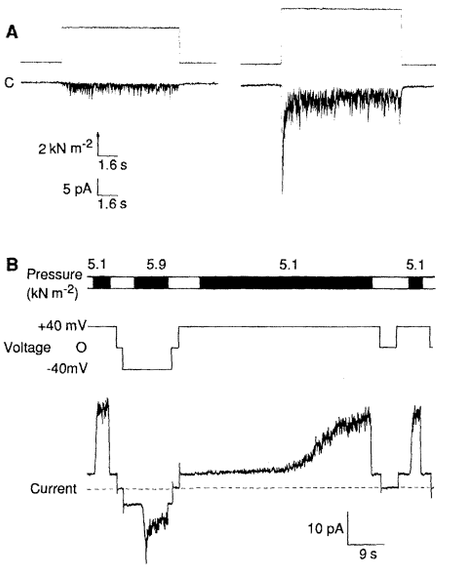

Fig. 4. Adaptation of MS channel activity to a sustained mechanical stimulus. (**A**) Pressure-dependent adaptation. Activation of MS channels by pressure pulses of different magnitude (left −3.7, right −5.7 kN m^{-2}, cell diameter = 5.3 μm). Top trace shows pressure, bottom trace, current. Pressure was applied with three-way valves (response time = 20 ms). The voltage was −40 mV. Solutions were 100 m*M* Cs^+ glutamate, 4 m*M* $MgCl_2$, 2 m*M* Na^+ adenosine 3′,5′-triphosphate (ATP), 2 m*M* Cs^+ EGTA, 250 m*M* mannitol, and 10 m*M* Cs^+, Hepes, *p*H 7.2 (pipette) and 100 m*M* Cs^+ glutamate, 2 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 210 m*M* mannitol, and 10 m*M* Cs^+ morpholinoethane sulfonic acid, *p*H 5.5 (bath). (**B**) Voltage-dependent adaptation. Top filled-in bar shows applied pressure, middle line shows applied voltage, and bottom trace shows currents (dashed line is the current level at 0 mV). Cell diameter was 5.2 μm. Pressure was applied by hand (response time = 1 s). Currents were identified as MS channel currents on the basis of pressure-dependence, selectivity (Fig. 2B), and sensitivity to gadolinium (*5, 6*). The threshold pressure for MS channel activation is lower at negative voltage than at positive voltage (*5*). This may in part explain the higher "bias" MS current observed at negative voltage. Solutions were the same as above except that ATP was omitted. The presence or absence of ATP had no effect on adaptation. Current traces (A and B) are from separate representative cells.

tion of the calculated tension, the currents for the three cells were normalized (Fig. 3B). Thus, yeast MS channels are regulated by membrane tension (*7a*). This regulatory mechanism of yeast MS channels may be similar to that of stretch-activated channels from animal cells (*8, 9*). The presence of tension-activated channels in yeast suggests that physiological membrane stretching could occur even in cells with mechanically resistant cell walls.

The mean tension that produced lysis in whole-cell recordings was between 6 to 11 mN m^{-1}. In comparison, the tension limit for phospholipid vesicles and erythrocytes is 3 to 4 (*10*) and 6 to 12 mN m^{-1} (*11*), respectively. The higher lytic tension of the erythrocyte membrane is attributed to a membrane-attached cytoskeleton (*11*). Because the tension limit of yeast spheroplasts is closer to that of erythrocytes than phospholipid vesicles, the existence of a parallel elastic element stabilizing the yeast plasma membrane is indicated. The form of the pressure-dependence of MS channel activation (and perhaps adaptation) may be determined by the mechanical properties of this parallel element. In preliminary experiments, we have observed that high internal, but not external, concentrations of Cl^- ($>10^{-1}M$) or Ca^{2+} ($>10^{-4}M$) halved the lytic tension in yeast spheroplasts. Since both ions destabilize cytoskeletal structures (*12*), this result suggests that the parallel elastic element is an internal cytoskeleton.

One feature of many mechanosensory systems is adaptation (or desensitization) to mechanical stimuli (*13*). At higher applied pressures, we found that MS channels displayed "adaptation"-like behavior (Fig. 4). At low pressure and at negative voltage in whole-cell recordings, MS channel activity remained fairly constant over the duration of the mechanical stimulus (Fig. 4A, left). If, however, pressure was increased further (and held constant), MS channel activity increased sharply and then relaxed to a new steady-state level (Fig. 4A, right).

Adaptation in yeast was voltage-dependent. Stepping up the pressure at +40 mV in a whole-cell recording resulted in a sustained increase in channel activity without adaptation (Fig. 4B, bottom trace, far left). This test condition, 5.1 kN m^{-2} pressure at +40 mV, was used to assess the adaptation status of MS channels. When 5.9 kN m^{-2} pressure was subsequently applied to the cell membrane at −40 mV, MS channels activated and then relaxed into an adapted state (Fig. 4B, bottom trace, near left). Upon reapplication of the test condition, MS channel activity was initially low, but gradually recovered (Fig. 4B, bottom trace, near right). On reapplication of pressure at positive voltage, the kinetics and final magnitude of the MS channel response had recovered (Fig. 4B, bottom trace, far right). The lack of adaptation at positive potentials has allowed the measurement of the maximum amount of pressure-activated current in whole-cell recordings (*5*). These results suggest a minimum number of MS channels per diploid cell (approximately 4 to 6 per square micrometer).

Adaptation of MS channels has also been observed in bullfrog saccular hair cells (*14*) and in fibroblasts (*15*). Three features of MS channel adaptation are shared by hair cells and yeast spheroplasts. Adaptation is voltage-dependent in both systems; adaptation is virtually absent at positive potentials (Fig. 4B) (*16*). The result of adaptation in hair cells is a shift in sensitivity rather than a change in the maximal response (*14*). In preliminary experiments, we have found that adaptation of yeast MS channels also had little effect on the maximal open probability. Instead, adaptation in yeast MS channels was due to a decrease in pressure sensitivity. More pressure was required to achieve the same open probability after adaptation had occurred (*5*). Finally, as in saccular hair cells (*14*), adaptation in yeast MS channels was a labile process. Adaptation in yeast was rarely observed in excised patches or in whole-cell recordings with high internal Ca^{2+} ($>10^{-4}M$) or Cl^- ($>10^{-1}M$).

The selectivity of MS channels indicates a possible physiological role in osmoregulation. Both cations and anions passed readily through MS channels. Osmotic stress on the cell could therefore be relieved by salt movement through MS channels. Second, MS channels may have a signaling function because they are the only Ca^{2+} channels that have been found in yeast. Channel-mediated influx of Ca^{2+} into cells and subsequent cytosolic Ca^{2+} increase is an important signaling mechanism in many eukaryotic cells (*17*). Furthermore, adaptation of a response to a sustained stimulus is a feature common to many cellular signaling mechanisms (*18*). Finally, MS channels may play a role in budding. Cell wall expansion and vesicle-mediated protein secretion are localized to the budding region of yeast cells (*19*). MS channel–mediated Ca^{2+} entry may be the etiological link between these processes. The discovery of MS channels in yeast, where powerful genetic and molecular approaches are available, presents an opportunity to learn more about the molecular mechanism of mechanosensation.

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7. Whole-cell recording mode was obtained by large suction applied to on-cell patches. This transition was marked by increases in capacitance (0.3 to 1.5 pF) and noise with only a small change in resistance. MS channel activity was elicited by positive, cell-inflating pressure applied to the inside of the cell.
7a. One implication of Laplace's law is that the larger the cell, the greater the sensivity to osmotic pressure changes. A possible function of MS channels in large cells, such as amphibian oocytes or erythrocytes [X. C. Yang and F. Sachs, *Biophys. J.* **51**, 252a (1987)], is to allow rapid solute movements and collapsing osmotic gradients, and to protect such cells against developing lytic tensions.
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21. The conductance measured in all solutions was that of the minimal conductance state. Judging by amplitude histogram analysis, we found all conductance states were approximate multiples of the minimal state. The minimum number of conductance states of the MS channels observed in excised patches was two (Fig. 1). The ion selectivity (determined from E_{rev} measurements) of both states appeared to be the same.
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Anticodon Switching Changes the Identity of Methionine and Valine Transfer RNAs

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The anticodon has previously been shown to play a role in recognition of certain transfer RNAs by aminoacyl-tRNA synthetases; however, the extent to which this sequence dictates tRNA identity is generally unknown. To investigate the contribution of the anticodon to the identity of *Escherichia coli* methionine and valine tRNAs, in vitro transcripts of these tRNAs were prepared that contained normal and interchanged anticodon sequences. Transcripts containing wild-type tRNA sequences were excellent substrates for their respective cognate aminoacyl-tRNA synthetases and were effectively discriminated against by a variety of noncognate enzymes. The mutant tRNAs produced by switching the anticodon sequences lost their original tRNA identity and assumed an identity corresponding to the acquired anticodon sequence. These results indicate that the anticodon contains sufficient information to distinguish methionine and valine tRNAs with high fidelity.

Attachment of the appropriate amino acid to each tRNA molecule is a crucial step in the translation of genetic information. The highly accurate aminoacylation of tRNAs is catalyzed by aminoacyl-tRNA synthetase enzymes specific for each of the 20 amino acids. The amino acid acceptor specificity, or identity, of each tRNA is determined by a particular set of structural features that allows recognition by one synthetase and excludes recognition by all others. The location of these identity elements remains largely unknown (*1*); however, significant progress has recently been made in determining structural features important for recognition of several tRNAs, including *Escherichia coli* alanine (*2, 3*), glutamine (*4*), phenylalanine (*5*), and serine (*6*) tRNAs and yeast $tRNA^{Phe}$ (*7, 8*). In addition, it has been known for some time that important recognition sites for aminoacyl-tRNA synthetases are present in the anticodons of certain tRNAs (*9*). Single base changes in the anticodon sequences of *E. coli* $tRNA_f^{Met}$ (recognizing RNA encoding the initiator methionine) (*10, 11*), $tRNA^{Trp}$ (*12*), $tRNA^{Arg}$ (*13*), and $tRNA^{Gly}$ (*14*), yeast $tRNA^{Val}$ (*15*), and beef $tRNA^{Trp}$ (*16*) have dramatic effects on aminoacylation of these tRNAs by their cognate synthetases. In addition, the specificity of amino acid acceptance of *E. coli* $tRNA^{Trp}$ (*12, 17*), *E. coli* $tRNA_f^{Met}$ (*18*), and yeast $tRNA^{Tyr}$ (*19*) is affected by alterations in the anticodon sequence. In only a few cases, however, has the quantitative contribution of the anticodon to tRNA identity been evaluated.

The CAU anticodon of methionine tRNAs (recognizing the codon AUG) is a crucial site of interaction with *E. coli* methionyl-tRNA synthetase (*10, 11*). To determine the extent to which the anticodon determines the identity of $tRNA^{Met}$, we have now transferred the methionine anticodon to a valine tRNA and examined the amino acid acceptor activity of the mutant $tRNA^{Val}$. In addition, we have prepared a methionine tRNA containing a valine anticodon and determined its aminoacylation specificity.

Wild-type and mutant methionine and valine tRNAs were prepared by in vitro transcription with T7 RNA polymerase from plasmids containing the appropriate tRNA genes, according to the methodology recently described by Sampson and Uhlenbeck (*7*). The genes were constructed from synthetic DNA oligonucleotides joined to a T7 RNA polymerase promoter; the promoter was positioned to initiate transcription with the 5′ terminal nucleotide of the mature tRNA. A Bst NI restriction enzyme site was included at the 3′ terminus of the gene to allow generation of a 3′ CCA end on the mature tRNA after run-off transcription from Bst NI–digested plasmid DNA. Normal 5′ phosphate termini were produced by inclusion of excess 5′ mononucleotide corresponding to the initiating nucleotide in the transcription reaction mixture (*7*). The in vitro transcripts obtained from plasmids carrying the genes for the elongator methionine tRNA ($tRNA_m^{Met}$) and for the major species of valine tRNA($tRNA_1^{Val}$) contain none of the modified bases normally found in the native tRNAs, but showed near normal amino acid acceptor activity with cognate aminoacyl-tRNA synthetases (Tables 1 and 2).

The anticodons of the methionine and valine tRNAs contain the same nucleotide bases in inverted order. Wild-type $tRNA^{Val}$ (anticodon UAC) is an extremely poor substrate for methionyl-tRNA synthetase. At high tRNA concentrations, the initial rate of methionine acceptance increases linearly with increasing $tRNA^{Val}$ (UAC) up to 40 μM, indicating that this concentration is far

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