Stadel, J. M., De Lean, A., & Lefkowitz, R. J. (1980) *J. Biol. Chem. 255*, 1436–1441.
Stadtman, T. C. (1961) *Enzymes, 2nd Ed. 5*, 55.
Stiles, G. L., Strasser, R. H., Lavin, T. N., Jones, L. R. Caron, M. G., & Lefkowitz, R. J. (1983) *J. Biol. Chem. 258*, 8443–8449.
Vauquelin, G., Geynet, P., Hanoune, J., & Strosberg, A. D. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3710–3714.
Voeikov, V., & Lefkowitz, R. J. (1980) *Biochim. Biophys. Acta 629*, 266–281.

Effect of Ethanol, Phenol, Formamide, Dimethyl Sulfoxide, Paromomycin, and Deuterium Oxide on the Fidelity of Translation in a Brain Cell-Free System†

Michael Laughrea,* Jacinthe Latulippe, and Anne-Marie Filion

ABSTRACT: The effects of six different agents (ethanol, phenol, formamide, dimethyl sulfoxide, heavy water, and a misreading-inducing antibiotic, paromomycin) on the activity and the accuracy of poly(U) translation have been compared under a range (2.5–12 mM) of Mg^{2+} concentrations in a rat brain cell-free system. The effect of most of these agents was remarkably sensitive to the Mg^{2+} concentration under which the assay was made. Ethanol decreased the fidelity of translation, and the efficiency of ethanol was increased 3–10-fold by higher Mg^{2+} concentrations. The effect of paromomycin was identical with that of ethanol, despite its very different structure. Formamide, a "RNA denaturant", increased the accuracy of translation under all Mg^{2+} concentrations tested. Dimethyl sulfoxide, another type of RNA denaturant, decreased the accuracy of translation under all Mg^{2+} concentrations tested. Phenol increased the accuracy of translation at high Mg^{2+} concentrations but decreased it at low Mg^{2+} concentrations. D_2O did not change to any appreciable extent the accuracy of translation, at all the Mg^{2+} concentrations used. There exists a cooperativity between the effects of Mg^{2+} and ethanol, Mg^{2+} and paromomycin, and Mg^{2+} and dimethyl sulfoxide on the fidelity of translation; no such cooperativity was detected between Mg^{2+} and formamide and between Mg^{2+} and D_2O. The differential effects of dimethyl sulfoxide and formamide are interpreted in terms of their different dielectric constants. The dielectric constant of dimethyl sulfoxide is higher than that of water, while that of formamide is lower.

The phenomenon of misreading mRNA has not been extensively studied in mammalian cell-free systems (Laughrea, 1981a). Nevertheless, it is widely believed that the accuracy of translation in mammalian systems is not markedly sensitive to environmental changes, in contrast to the high sensitivity of bacterial systems (Schlanger & Friedman, 1973; Burrans & Kurtz, 1977; Kurtz, 1979). This belief is implicitly based on the assumption that the effect of an environmental change (e.g., temperature, pH, organic solvents, etc.) is independent of the ionic conditions under which it is studied. In only two instances were both prokaryotic and mammalian systems compared under identical ionic conditions; the data were made largely inconclusive by the presence of a high level of endogenous RNA directed incorporation in the poly(U)-directed cell extracts (Laughrea, 1981b).

In this paper, we investigated some parameters of the translation fidelity in a rat brain cell-free extract directed by poly(U). The effects of six different agents (ethanol, phenol, formamide, dimethyl sulfoxide, deuterium oxide, and a misreading-inducing antibiotic, paromomycin) on the activity and the accuracy of translation were compared under a range of Mg^{2+} concentrations. The experimental results indicate that the effect of most of these agents is remarkably sensitive to the Mg^{2+} concentration under which the assay is made, that ethanol and dimethyl sulfoxide decrease the accuracy of translation, that formamide and phenol can increase the accuracy of translation, and that the presence of deuterium oxide does not affect the fidelity of translation.

Materials and Methods

Animals. Male rats, either Sprague-Dawley or Fisher 344, were used. No significant difference was seen between extracts from either breed.

Chemicals. Poly(U), ATP (disodium salt), GTP (disodium salt), creatine phosphate (disodium salt), calf liver tRNA, tRNA from brewers' yeast specific for phenylalanine ($tRNA^{Phe}$), all enzymes, and antibiotics were purchased from Boehringer Mannheim. Paromomycin sulfate was a gift from Dr. J. J. Brossard, Parke-Davis, Canada. Spermine tetrahydrochloride and spermidine trihydrochloride were from Sigma. Deuterium oxide (D_2O) was purchased from Merck Sharp & Dohme, Canada. Radioactive [^{3}H]leucine (40–60 Ci/mmol) and [^{14}C]phenylalanine (>450 mCi/mmol) were purchased from both Amersham and New England Nuclear. Some of the [^{3}H]leucine batches contained, on arrival, higher than specified levels of impurities, resulting in a doubling or even a tripling of the background incorporation. These batches were discarded or, later in the study, purified by paper chromatography using 25:4:10 1-butanol:acetic acid:water or 7:7:6 pyridine:isoamyl alcohol:water as the solvent system. The discarded impurities were more toluene soluble than leucine itself. Most of them moved faster than leucine, but both faster moving and slower moving impurities contributed to the background incorporation. All acceptable [^{3}H]leucine batches

† From the Lady Davis Institute for Medical Research of the Sir Mortimer B. Davis—Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2. *Received June 3, 1983.* This work was supported by grants from the Medical Research Council of Canada and the Conseil de la Recherche en Santé du Québec. M.L. is a recipient of a research career development award from the Conseil de la Recherche en Santé du Québec.

0006-2960/84/0423-0753$01.50/0

were used within 1 month. No impurity could be detected in the [^{14}C]phenylalanine batches. All other chemicals were of the highest purity commercially available. Phenol was redistilled before use.

Buffers. Buffer A contained 7.5 mM potassium phosphate, 0.3 mM spermine, 0.4 mM spermidine, 50 mM KCl, 50 mM potassium acetate, 5 mM NH_4Cl, 5 mM magnesium acetate, and 1 mM dithiothreitol, pH 7.5. The homogenization buffer was buffer A with 0.25 M sucrose–20 mM *N*-[tris(hydroxymethyl)methyl]glycine (Tricine) (pH 7.5) and without phosphate.

Cell-Free Extracts. Brains were quickly removed and rinsed in ice-cold homogenization buffer. All procedures were performed at 0–4 °C unless otherwise noted. The brains were homogenized (0.5 g wet weight/mL of homogenization buffer) with 6 strokes of a Dounce homogenizer (Bellco). The homogenate was centrifuged at 2500*g* for 3 min after which the supernatant was centrfuged at 20000*g* for 40 min. The resulting cell-free extract was preincubated for 60 min at 37 °C in homogenization buffer containing 1 mM neutralized GTP, 1 mM neutralized ATP, 10 mM creatine phosphate, 50 μg/mL creatine kinase, 50 μM amino acids, and 10 μM puromycin. This was followed by dialysis against buffer A, centrifugation for 10 min in the Eppendorf microcentrifuge, separation of the supernatants (S20) into aliquots, and storage in liquid N_2.

Poly(U) Translation. The basic reaction mixture contained, in 100 μL, 25 μL of S20 containing 2–3 pmol of ribosomes depending on the preparation, 5 μg of creatine kinase, 10 mM creatine phosphate, 1 mM ATP, 1 mM GTP, 7.5 mM dithiothreitol, 10 μg of unfractionated calf liver tRNA, 10 μg of $tRNA^{Phe}$, 160 μg of poly(U), 10.6 μCi of neutralized [^{3}H]leucine, 27.5 μM leucine, 0.04 μCi of [^{14}C]phenylalanine, 110 μM phenylalanine, 40 μM each of all other amino acids, 1 μL of 0.1 N KOH, 7.5 mM potassium phosphate, 0.35 mM spermine, 0.45 mM spermidine, 55 mM KCl, 55 mM potassium acetate, 5 mM NH_4Cl, 0.4 mM $CaCl_2$, and from 2.5 to 12 mM magnesium acetate depending on the experiment. The final pH was 7.4. The incorporation increased linearly for more than 60 min, at which time it was stopped by the addition of 3 mL of 5% trichloroacetic acid containing 1% casamino acids. The samples were further processed as described previously (Laughrea, 1982). Care was taken to rinse the funnel of the filter holder and especially the edges of the filter after removal of the funnel. Amino acid incorporation in the absence of poly(U) was resistant to puromycin and cycloheximide, indicating that it was not ribosome directed. The backgrounds from blank samples [i.e., complete mixtures minus poly(U)] were less than 5 cpm above the machine background in the ^{14}C channel and approximately 200 cpm above the machine background in the ^{3}H channel. Total precipitable ^{3}H radioactivity in the presence of poly(U) was always greater than 350 cpm and usually lower than 2500 cpm in the experiments described below. The incorporated radioactivity reported below was obtained by subtracting the results in the absence of poly(U) from the results in the presence of poly(U).

Results

Ethanol Decreases the Fidelity of Mammalian Translation. Figure 1 shows the effect of ethanol concentration on the fidelity of poly(U) translation in a rat brain extract. The experiment was performed at 6 mM Mg^{2+} and under ionic conditions which should approximate the in vivo ones (Tower, 1969; Wickson-Ginzburg & Solomon, 1963; Raina & Jänne, 1975; Dratz & Handler, 1952; Rapoport, 1945). It is clear that ethanol reduced the accuracy of poly(U) translation under these conditions. Similar results were also obtained with a rat liver extract.

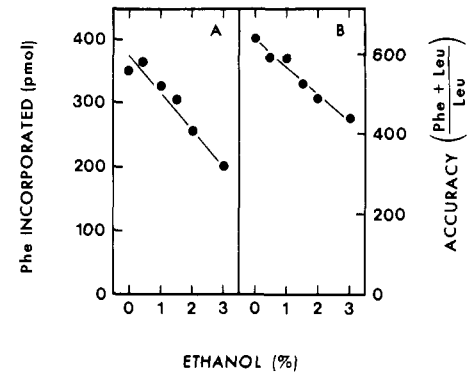

FIGURE 1: Effect of ethanol on the rate (A) and the fidelity (B) of poly(U) translation. A rat brain S20 was assayed for poly(U) translation as described under Materials and Methods. The accuracy of the incorporation is expressed as (Phe incorporated + Leu incorporated)/(Leu incorporated). The assay was done at 6 mM magnesium acetate.

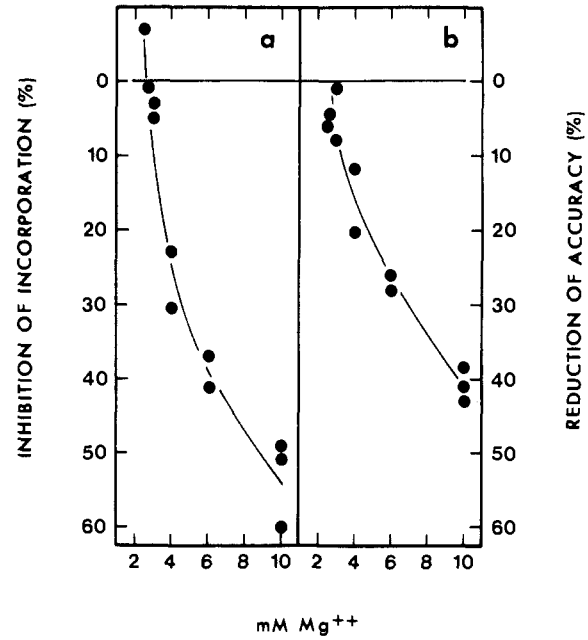

FIGURE 2: Effect of 2.4% ethanol on the rate of incorporation (A) and the fidelity of translation (B) at various Mg^{2+} concentrations. A rat brain S20 was assayed as described in Figure 1. A value above the 0 line means that 2.4% ethanol has a stimulatory effect. A value below the 0 line means that 2.4% ethanol has an inhibitory effect. At 4 mM Mg^{2+}, the incorporation activity was 126 phenylalanines per ribosome, and the accuracy was 660.

Activity and accuracy measurements were also made under a variety of Mg^{2+} concentrations (Figure 2). At 2.5–3 mM Mg^{2+}, the fidelity of translation was hardly affected by a concentration of 2.4% ethanol; at 10 mM Mg^{2+}, it was reduced by 40%. The rate of incorporation was also inhibited by ethanol in a similar Mg^{2+}-dependent fashion (Figure 2a). Other experiments have shown that 2.4% ethanol at 8 mM Mg^{2+} has the same inhibitory effect as 7% ethanol at 2.75 mM Mg^{2+} and that 2.4% ethanol at 2.75 mM Mg^{2+} has the same inhibitory effect as 0.2–0.4% ethanol at 10 mM Mg^{2+}. Thus, our in vitro system is 3–10 times less sensitive to ethanol at low Mg^{2+} concentrations than at high Mg^{2+} concentrations.

Paromomycin Mimics Ethanol. Paromomycin is a misreading-inducing amino glycoside drug whose structure bears no resemblance to that of ethanol (Wilhelm et al., 1978a). Yet Figure 3 shows that the effects of paromomycin concentration

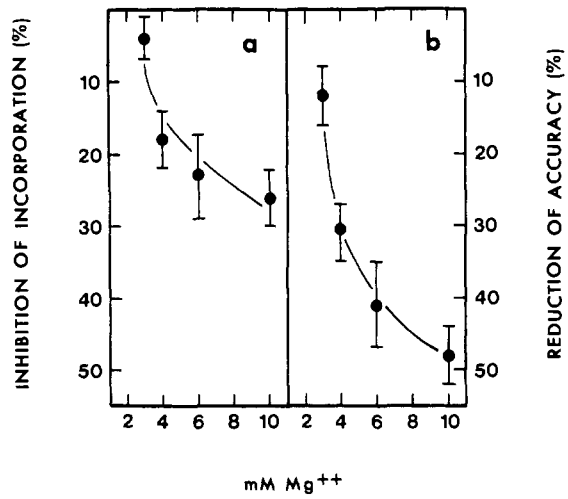

FIGURE 3: Effect of 160 μM paromomycin (100 μg/mL) on the rate of incorporation (A) and the fidelity of translation (B) at various Mg^{2+} concentrations. Conditions are as described in the legend of Figure 2. The error bars represent the standard errors of the mean of three different experiments. At 4 mM Mg^{2+}, the average incorporation activity was 112 phenylalanines per ribosome, and the accuracy was 700.

on the accuracy and the rate of incorporation are qualitatively identical with those of ethanol. The effect of 160 μM paromomycin on the accuracy of translation was indistinguishable from that of 3.5% ethanol, no matter the Mg^{2+} concentration used; its effect on the activity was not different from that of 1.5% ethanol (data not shown). Ethanol, if used at concentrations higher than 3.5%, induced more misreading than paromomycin could (data not shown). On the other hand, paromomycin was more effective at inducing misreading than ethanol: a given loss of accuracy was accompanied by a smaller loss in activity when paromomycin, rather than ethanol, was used. This was independent of the ethanol concentration (0–5%), the Mg^{2+} concentration (3–10 mM), and the paromomycin concentration provided it was 160 μM or less (Figures 2 and 3 and data not shown). Paromomycin concentrations higher than 160 μM failed to further decrease the accuracy but did further reduce the rate of incorporation (data not shown).

Phenol and Formamide Differ Drastically from Ethanol. If such widely different compounds as ethanol and paromomycin have similar effects, it may be thought that moderately analogous molecules such as ethanol and phenol will have similar effects on the accuracy and the rate of incorporation. The open circles in Figure 4 show that this is not the case. Phenol inhibited the activity to a greater extent at *low* Mg^{2+} concentrations than at high Mg^{2+} concentrations. Furthermore, it *increased* the fidelity of translation at high Mg^{2+} concentrations, had no effect at 6 mM Mg^{2+}, and decreased it at low Mg^{2+} concentrations.

These unexpected differences prompted us to study some other solvents in order to get a better perspective about the way the accuracy of protein synthesis is influenced by the environment. Formamide is a good denaturant for nucleic acids (Pinder et al., 1974; Helmkamp & Ts'o, 1961; Levine et al., 1963; Herskovits, 1962). It increased the fidelity of translation under all conditions tested [Figure 4b, (●)]. The degree of stimulation was also independent of the Mg^{2+} concentration. However, formamide decreased the activity at low Mg^{2+} concentration and increased it at high Mg^{2+} concentration [Figure 4a, (●)].

Effect of Dimethyl Sulfoxide and Deuterium Oxide. Dimethyl sulfoxide (Me_2SO) is also a good denaturant for nucleic

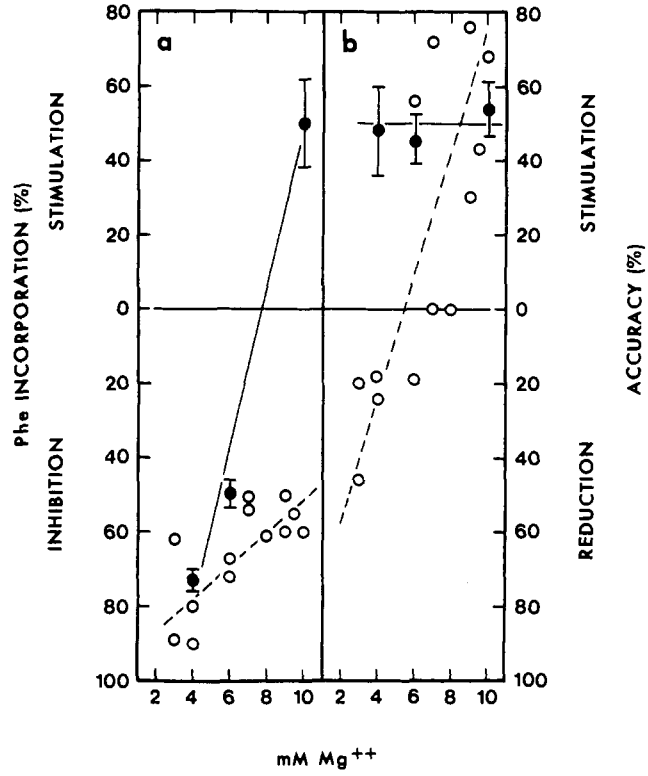

FIGURE 4: Effect of 0.45% phenol (○) and 5% neutralized formamide (●) on the rate of incorporation (A) and the fidelity of translation (B) at various Mg^{2+} concentrations. Conditions are as described in the legend of Figure 3. At 4 mM Mg^{2+}, the average incorporation activity was 115 phenylalanines per ribosome, and the accuracy was 640.

Table I: Effect of Me_2SO and D_2O on the Incorporation and the Fidelity of Translation at Two Different Mg^{2+} Concentrations[a]

$[Mg^{2+}]$ (mM)	D_2O (%)	Me_2SO (%)	incorporation (%)	accuracy (%)
4	0	0	100	100
4	0	4	72.6 ± 6	72 ± 8
4	34	0	100 ± 10	103 ± 15
10	0	0	100	100
10	0	4	50 ± 5	44 ± 7
10	34	0	35 ± 5	98 ± 5

[a] Conditions are as described in the legend of Figure 1. At 4 mM Mg^{2+}, 100% corresponds to an incorporation of 120 amino acids per ribosome and an accuracy of 700 and at 10 mM Mg^{2+} to an incorporation of 29 amino acids per ribosome and an accuracy of 350. The errors are standard errors of the mean of three experiments. The definition of accuracy is as described in the legend of Figure 1.

acids (Strauss et al., 1968; Birnboim, 1972; Helmkamp & Ts'o, 1961; Herskovits, 1962). Its effect on the accuracy and the rate of incorporation was similar to that of ethanol but very different from that of formamide (Table I).

Finally, D_2O can increase by 20 °C the melting temperature of salmon sperm DNA and of the poly(U)–poly(A) complex (Lewin, 1966). Will it reduce the accuracy of translation through stabilization of the short codon–anticodon double helix? Table I shows that no matter the Mg^{2+} concentration, 34% D_2O had no detectable effect on the fidelity of poly(U) translation. On the other hand, it reduced the rate of incorporation by 65% at 4 mM Mg^{2+} and left it unchanged at 10 mM Mg^{2+}.

Discussion

Effect of Ethanol and Paromomycin. Previous studies on spleen and reticulocyte extracts had suggested that the fidelity

of poly(U) translation was increased or left unchanged in the presence of low concentrations of ethanol (Friedman et al., 1968; Stavy, 1968; Weinstein et al., 1966). This seemingly disagrees with our finding that ethanol decreases the fidelity of translation. However, two comments are in order. First, we note that with the Mg^{2+} conditions under which Stavy (1968) attempted to measure the effect of ethanol, no poly(U)-dependent leucine uptake by preincubated ribosomes could be detected in the absence of ethanol. Thus, Stavy's work on the effect of ethanol should have been done at a higher Mg^{2+} concentration, where leucine uptake was detectable, to be conclusive and comparable to our experiments. Second, the experiments of Friedman et al. (1968) and Weinstein et al. (1966) were performed under conditions where very high endogenous RNA directed leucine incorporation occurred. They did not check to see whether poly(U) influenced directly its level or whether this influence was ethanol dependent (Laughrea, 1981b). As a result, their data are consistent with either a decrease or an increase of fidelity in the presence of ethanol, depending on whether or not ethanol increases the inhibitory potency of poly(U) toward endogenous leucine incorporation. To be conclusive, their work should have been done with ribosomes having less endogenous incorporation.

Our experiments are the only ones to provide relatively unambiguous data on the effect of ethanol on the fidelity of translation in a mammalian system. Taken together with experimental results on *Escherichia coli* (So & Davie, 1964; Gallant & Prothero, 1980; Phoenix et al., 1983) and yeast (Schlanger & Friedman, 1973), they suggest that ethanol decreases the fidelity of translation in all living systems and that, contrary to previous beliefs [e.g., see Kurtz (1979), Burrans & Kurtz (1977), and Vazquez (1979)], there are no qualitative differences between the responses of bacterial and mammalian ribosomes to ethanol and, perhaps, to any other organic solvent. Furthermore, our results reveal that the effect of ethanol is exquisitely sensitive to the Mg^{2+} concentration in the assay mixture. A quantitative comparison between the effect of ethanol on bacterial and mammalian systems is uninterpretable unless it is done under identical ionic conditions. For example, So & Davie (1964) have observed in *E. coli* extracts that 2.4% ethanol reduces the accuracy of poly(U) translation by 85%. This inhibition is stronger than that which we obtain in rat brain. Given that the two sets of experiments were performed under very different ionic conditions, one should not conclude that there are quantitative differences between the responses of mammalian and bacterial cell-free extracts to ethanol.

Wallace et al. (1973) had shown that the lower the Mg^{2+} concentration, the more the amino acid incorporation from endogenous *E. coli* polysomes was inhibited by streptomycin, a misreading-inducing amino glycoside in bacterial systems. In contrast, our results with ethanol indicated that the ***higher*** the Mg^{2+} concentration, the more ethanol inhibited phenylalanine incorporation. This, and the fact that misreading-inducing antibiotics have a structure and a site of action different from ethanol, suggested that they may give rise to a different inhibition pattern than that of ethanol. This was our rationale for the study of paromomycin, a drug related in structure to streptomycin and which decreases the accuracy of translation in mammalian systems (Wilhelm et al., 1978b). We found that the effect of paromomycin on the fidelity and the rate of incorporation had a pattern of Mg^{2+} concentration dependence identical with that of ethanol. Paromomycin also induces misreading in extracts from yeast (Singh et al., 1979), wheat embryo (Wilhelm et al., 1978a), tetrahymena (Palmer & Wilhelm, 1978), and cultured human cell extracts (Wilhelm et al., 1978b). However, the effect of paromomycin on rat brain extracts is not as great as reported in any of these other systems.

Effect of Formamide and Me_2SO. It is of interest to note that the effects of formamide and Me_2SO, two "RNA denaturants", on the rate and the accuracy of incorporation were radically different. (i) Formamide increased accuracy; Me_2SO decreased it. (ii) Formamide could increase or decrease the rate of translation depending on the Mg^{2+} concentration; Me_2SO always decreased the rate of translation.

It is possible to explain the stimulatory effect of formamide on the fidelity and on the rate of incorporation at high Mg^{2+} concentrations. Formamide is well-known as a hydrogen-bond breaker thanks, among other things, to a dielectric constant about 40% *larger* than that of water (Pinder et al., 1974; Herskovits, 1962). Consequently, it should strengthen the tRNA–solvent and mRNA–solvent hydrogen bonds and thus destabilize the aminoacyl-tRNA–codon (aa-tRNA–codon) complex; this favors the rejection of weak base pairs and thus a higher fidelity of translation, according to the kinetic tuning models of the speed and accuracy of protein synthesis [reviewed in Laughrea (1981a)]. Mg^{2+} is an agent which stabilizes the aa-tRNA–codon–ribosome complex (Gluckhova et al., 1975). Thus, high Mg^{2+} concentrations inhibit the translocation step (Gavrilova et al., 1976) and reduce the rate of incorporation. Formamide counteracts this excess stabilization and therefore stimulates translocation. At Mg^{2+} concentrations lower than those optimal for proper stabilization of the aa-tRNA–ribosome–codon interaction, formamide will further destabilize the complex and reduce the rate of incorporation.

Even though Me_2SO is a good RNA denaturant (Strauss et al., 1968; Birnboim, 1972; Helmkamp & Ts'o, 1961), it differs from formamide in several structural respects. (i) Its dielectric constant is about 40% lower than that of water (Herskovits, 1962); (ii) it is an aprotic solvent and thus has a tendency to accept rather than donate protons, in contrast to formamide which can both accept and donate protons (Rammler & Zaffaroni, 1967; MacGregor, 1967); (iii) because of its two methyl groups, it should be a better breaker of "hydrophobic bonds" (e.g., base stacking) than formamide. One or several of these structural properties could be the reason why Me_2SO has effects contrary to those of formamide on the fidelity of translation. The fact that ethanol and Me_2SO share properties (i) and (iii) (the dielectric constant of ethanol is 24), and that both are poorer proton donors than H_2O, is consistent with our observation that they have similar effects on the rate and the fidelity of incorporation. One may imagine that the lower dielectric constants of ethanol and Me_2SO stabilize mRNA–tRNA interactions and therefore favor miscoding [it has been shown experimentally that ethanol stabilizes the aa-tRNA–codon–ribosome complex (Gluckhova et al., 1975)]. Furthermore, since the low dielectric constants of ethanol and Me_2SO reduce the solvation of the Mg^{2+} ions, a more effective neutralization of the phosphate backbones of the codon–anticodon complex could result. This reduction of the repulsion between the phosphates would further stabilize the codon–anticodon interaction and increases the miscoding. Whether this solvation effect is connected to the cooperativity observed between Mg^{2+} and ethanol, and Mg^{2+} and Me_2SO, is not immediately apparent, especially since no cooperativity was detected between Mg^{2+} and formamide.

Effect of Phenol and Deuterium Oxide. The effect of phenol on the fidelity of translation is perhaps the most in-

triguing of our results. At high Mg^{2+} concentrations, phenol behaved like formamide: it increased the accuracy. At low Mg^{2+} concentrations, it behaved like Me_2SO and ethanol: it decreased the accuracy. At the structural level, phenol has a low dielectric constant and a bulky hydrophobic group, like ethanol and Me_2SO; it can both give and accept protons, like ethanol and formamide. Why then does it not behave like ethanol? Phenol does differ from ethanol by the structure of its hydrophobic group which is an aromatic ring. At low Mg^{2+} concentrations (or under any conditions of weak codon–anticodon interactions), the effect of phenol's low dielectric constant would dominate, and it would then behave roughly like ethanol and Me_2SO. At high Mg^{2+} concentrations (or under any conditions of strong codon–anticodon interactions), the codon–anticodon "double helix" would adopt a conformation or a rigidity which favors the preferential insertion of phenol between the bases of poly(U) or of the tRNA anticodon. Such an insertion could disrupt or weaken some hydrogen bonds in the codon–anticodon interaction and therefore promote greater accuracy [reviewed in Laughrea (1981a)].

Rats, mice, and dogs become dangerously ill when the concentration of D_2O in the body fluid reaches 30% (Katz, 1960; Furness, 1960). The melting temperature of salmon sperm DNA and of the poly(A)–poly(U) complex is about 20 °C higher in D_2O at 10^{-3} M ionic strength (Lewin, 1966). Thus, it was plausible to expect D_2O to reduce the accuracy of translation through stabilization of the short codon–anticodon double helix. This possibility did not materialize. No matter the Mg^{2+} concentration, we found that 34% D_2O had no effect on the fidelity of translation. This does not contradict the findings of Lewin (1966) since at the ionic strength at which our incorporation experiments were done, Mahler et al. (1963) had been unable to detect any difference in the melting temperature of four different RNAs in H_2O or D_2O solutions.

Generalities. The effect of formamide, phenol, Me_2SO, and D_2O on the fidelity of translation had never been studied previously, either on eukaryotes or on prokaryotes. The effect of other environmental changes, such as pH (Lamfrom & Grunberg-Manago, 1967) and ethanol (Stavy, 1968; Friedman et al., 1968), paromomycin (Wilhelm et al., 1978a,b; Butzow et al., 1981), and spermidine (Higarashi et al., 1982) concentrations, had been studied to some extent in mammalian extracts but, like related studies in prokaryotes (So & Davie, 1964; Grunberg-Manago & Dondon, 1965; Szer & Ochoa, 1964; Laughrea, 1981c; Phoenix et al., 1983), only at one Mg^{2+} concentration. Our results indicate that with four agents out of six (ethanol, Me_2SO, phenol, and paromomycin) it is erroneous to assume a Mg^{2+}-independent effect on the fidelity of translation. For example, low concentrations of phenol can yield diametrically opposite effects depending on whether the fidelity measurement is made at low or high Mg^{2+} concentrations. All six agents studied showed different influences on amino incorporation at different Mg^{2+} concentrations.

If the effects of most of the six agents studied in this paper depend strongly on the Mg^{2+} concentration, could they not as well depend strongly on the concentration of anything else? We do not have the answer to this question, but as a result of other experiments performed at pH 8.3 or in the absence of added $tRNA^{Phe}$, we do know the following. The effects of 2.4% ethanol and 5% formamide on the fidelity of translation at pH 8.3 or in the absence of added $tRNA^{Phe}$ were analogous to their effects at pH 7.4 (Figures 2 and 4). On the other hand, 2.4% ethanol stimulated the activity at less than 5 mM Mg^{2+} in the absence of added $tRNA^{Phe}$ (data not shown). Ethanol should be expected to stimulate poly(U) translation under conditions which are unfavorable for initiation, such as low concentrations of Mg^{2+} and $tRNA^{Phe}$ (Laughrea, 1981a). Furthermore, in the absence of added $tRNA^{Phe}$, which reduced the incorporation activity by as much as 65%, the fidelity of translation was increased by 250–300% in the presence of 0.37% phenol at 10 mM Mg^{2+} and left unchanged at 3.5 mM Mg^{2+} (compare with Figure 4). Thus, it is correct to assume that the effect of ethanol and formamide on the fidelity of translation is independent of pH and $tRNA^{Phe}$ concentration. Quantitative differences were found, however, in the case of phenol.

We are aware that organic solvents can interact with proteins. However, given that the structure of proteins is, in general, much less affected by low concentrations of organic solvents than the structure of double-stranded nucleic acids (Gordon & Jencks, 1963; Levine et al., 1963; Tanford, 1968), it seems reasonable to assume that, to a first order of approximation, the solvent changes which we have studied in this paper influence the fidelity of translation predominantly by acting directly at the level of the short codon–anticodon double strand.

Finally, the accuracy of poly(U) translation in our in vitro system was on the order of 700. As pointed out previously (Gavrilova et al., 1981), there are no factual grounds to assert that this is lower than the rate of Phe-Leu substitutions during translation in vivo. Indeed, our poly(U)-directed leucine misincorporation measurments should include all frame-shift errors (Weiss & Gallant, 1983) caused by the initial misincorporation and all errors resulting in dissociation of peptidyl-tRNA from ribosomes (Caplan & Menninger, 1979). Moreover, it is the sum of two misreadings, one at the 5′ codon base and another at the 3′ codon base. Had we only been studying the misreading of UUU as, e.g., CUU and had we been excluding all misreading events which caused a frameshift error or dissociation of peptidyl-tRNA, we would have measured a lower misreading level. This could have been more appropriately compared to available in vivo measurements (Loftfield & Vanderjagt, 1972; Edelman & Gallant, 1977), with the caveat that these in vivo experiments depended on the implicit assumption that the missynthesized protein measured was as stable as the normal protein. Such an assumption is questionable at best (Schaeffer, 1973; Zipser & Bhavsar, 1976; Bergquist & Truman, 1978) and may have led to estimations of in vivo misreading rates which are significantly lower than the true rate of misreading at the level of the nascent polypeptide chain. On the contrary, our poly(U) experiments, done under reasonably physiological conditions, may provide a better estimate of the in vivo rate of misreading UUU in a context of uridines than any of the presently available in vivo data.

We are also aware that no other eukaryotic in vitro system under the direction of poly(U) is more active than ours.

Added in Proof

In the absence of $tRNA^{Phe}$, 2.4% ethanol inhibited the accuracy by 58 (±2)% at 3.5 mM Mg^{2+} and by 57 (±2)% at 10 mM Mg^{2+}, i.e., even more than in the presence of added $tRNA^{Phe}$.

Acknowledgments

We are indebted to Drs. L. Brakier-Gingras, C. Côté, and L. Pinsky for critical reading of the manuscript, to H. Karam for typing the manuscript, and to C. Lalonde for the illustrations. Sprague-Dawley rats were a gift from Dr. Claude Côté.

Registry No. Mg, 7439-95-4; D_2O, 7789-20-0; ethanol, 64-17-5;

phenol, 108-95-2; formamide, 75-12-7; dimethyl sulfoxide, 67-68-5; paromomycin, 7542-37-2; poly(U), 27416-86-0.

References

Bergquist, P. L., & Truman, P. (1978) *Mol. Gen. Genet. 164*, 105–108.

Birnboim, H. C. (1972) *Biochemistry 11*, 4588–4591.

Burrans, L., & Kurtz, D. I. (1977) *J. Mol. Biol. 112*, 349–352.

Butzow, J. J., McCool, M. G., & Eichhorn, G. L. (1981) *Mech. Ageing Dev. 15*, 203–216.

Caplan, A. B., & Menninger, J. R. (1979) *J. Mol. Biol. 134*, 621–637.

Dratz, A. F., & Handler, P. (1952) *J. Biol. Chem. 197*, 419–431.

Edelman, P., & Gallant, J. (1977) *Cell (Cambridge, Mass.) 10*, 131–137.

Eichhorn, G. L., Berger, N. A., Butzow, J. J., Clark, P., Heim, J., Pitha, J., Richardson, C., Rifkind, J. M., Shin, Y., & Tarien, E. (1973) *Adv. Exp. Med. Biol. 40*, 43–66.

Friedman, S. M., Berezney, R., & Weinstein, I. B. (1968) *J. Biol. Chem. 243*, 5044–5048.

Furness, F. N. (1960) *Ann. N.Y. Acad. Sci. 84*, 573–781.

Gallant, J. A., & Prothero, J. (1980) *J. Theor. Biol. 83*, 561–578.

Gavrilova, L. P., Perminova, I. N., & Spirin, A. (1981) *J. Mol. Biol. 149*, 69–78.

Gluckhova, M. A., Belitsina, N. V., & Spirin, A. S. (1975) *Eur. J. Biochem. 52*, 197–202.

Gordon, J. A., & Jencks, W. P. (1963) *Biochemistry 2*, 47–57.

Grunberg-Manago, M., & Dondon, J. (1965) *Biochem. Biophys. Res. Commun. 18*, 517–522.

Helmkamp, G. K., & Ts'o, P. O. P. (1961) *J. Am. Chem. Soc. 83*, 138–142.

Herskovits, T. T. (1962) *Arch. Biochem. Biophys. 97*, 474–484.

Higarashi, K., Hashimoto, S., Miyake, A., Kashiwagi, K., & Hirose, S. (1982) *Eur. J. Biochem. 128*, 597–604.

Katz, J. J. (1960) *Sci. Am. 203*, 106–116.

Kurtz, D. I. (1979) *Gerontology (Basel) 25*, 228–230.

Lamfrom, H., & Grunberg-Manago, M. (1967) *Biochem. Biophys. Res. Commun. 27*, 1–6.

Laughrea, M. (1981a) *Biochimie 63*, 145–168.

Laughrea, M. (1981b) *Gerontology (Basel) 27*, 158–160.

Laughrea, M. (1981c) *Can. J. Biochem. 59*, 799–801.

Laughrea, M. (1982) *Biochemistry 21*, 5694–5700.

Levine, L., Gordon, J. A., & Jencks, W. P. (1963) *Biochemistry 2*, 168–175.

Lewin, S. (1966) *Arch. Biochem. Biophys. 115*, 62–66.

Loftfield, R. B., & Vanderjagt, D. (1972) *Biochem. J. 128*, 1353–1356.

MacGregor, W. S. (1967) *Ann. N.Y. Acad. Sci. 141*, 3–12.

Mahler, H. R., Dutton, G., & Mehrotra, B. D. (1963) *Biochim. Biophys. Acta 68*, 199–210.

Palmer, E., & Wilhelm, J. M. (1978) *Cell (Cambridge, Mass.) 13*, 329–334.

Phoenix, P., Melancon, P., & Brakier-Gingras, L. (1983) *Mol. Gen. Genet. 189*, 123–128.

Pinder, J. C., Stayvow, D. Z., & Gratzer, W. B. (1974) *Biochemistry 13*, 5369–5373.

Raina, A., & Janne, J. (1975) *Med. Biol. 53*, 121–147.

Rammler, D. H., & Zaffaroni, A. (1967) *Ann. N.Y. Acad. Sci. 141*, 13–23.

Rapoport, S. (1945) *J. Biol. Chem. 161*, 429–435.

Schaeffer, J. R. (1973) *J. Biol. Chem. 248*, 7473–7480.

Schlanger, G., & Friedman, S. M. (1973) *J. Bacteriol. 115*, 129–138.

Singh, A., Ursic, D., & Davies, J. (1979) *Nature (London) 277*, 146–148.

So, A. G., & Davie, E. W. (1964) *Biochemistry 3*, 1165–1169.

Stavy, L. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 347–353.

Strauss, J. H., Kelly, R. B., & Sinsheimer, R. L. (1968) *Biopolymers 6*, 793–807.

Szer, W., & Ochoa, S. (1964) *J. Mol. Biol. 8*, 823–834.

Tanford, C. (1968) *Adv. Protein Chem. 23*, 121–280.

Tower, D. B. (1969) *Handbook of Neurochemistry*, Vol. I, pp 1–24, Plenum Press, New York.

Vazquez, D. (1979) *Mol. Biol., Biochem. Biophys. 30*, 1–307.

Wallace, B. J., Tai, P. C., Herzog, E. L., & Davis, B. D. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1234–1237.

Weinstein, I. B., Friedman, S. M., & Ochoa, M. (1966) *Cold Spring Harbor Symp. Quant. Biol. 31*, 671–680.

Weiss, R., & Gallant, J. (1983) *Nature (London) 302*, 389–393.

Wickson-Ginzburg, M., & Solomon, A. K. (1963) *J. Gen. Physiol. 46*, 1303–1315.

Wilhelm, J. M., Pettitt, S. E., & Jessop, J. J. (1978a) *Biochemistry 17*, 1143–1149.

Wilhelm, J. M., Jessop, J. J., & Pettitt, S. E. (1978b) *Biochemistry 17*, 1149–1153.

Zipser, D., & Bhavsar, P. (1976) *J. Bacteriol. 127*, 1538–1542.