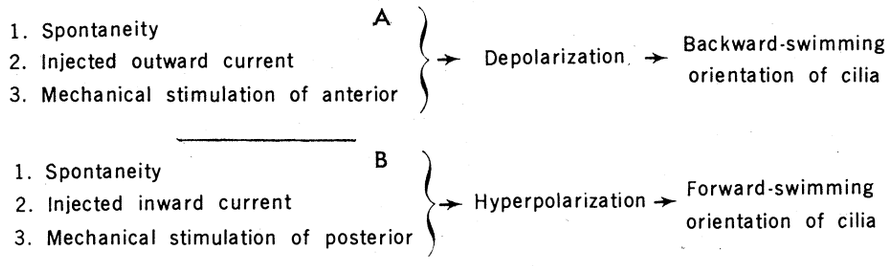

Fig. 4. Summary of bioelectric events which influence the locomotor behavior of *Euplotes*. Events A1 and B1 are illustrated in Fig. 1, A_1 and A_2. Events A2 and B2 are illustrated in Fig. 1, B_1 and B_2. Event A3 is illustrated in Fig. 2, B and C, while event B3 is illustrated in Fig. 2, D and E.

We examined electrical responses to mechanical stimulation (*5*). The depolarizations were evoked by a light tap applied to the anterior end of the specimen with a fine glass stylus driven by a ceramic phono cartridge (Fig. 2, B and C). Hyperpolarizations were evoked by a similar stimulus applied to the posterior end (Fig. 2, D and E). As in *Paramecium* (*5*), mechanical stimulation at the anterior end invariably results in a depolarization, and stimulation at the posterior end always elicits a hyperpolarization.

The ciliary organelles of *Euplotes* invariably assume an anterior-pointing (reversed) orientation in response to depolarization (Fig. 2, B and C) and an augmented posterior-pointing (forward locomotion) orientation in response to hyperpolarization (Fig. 2, D and E). This correlation between membrane potential and the orientation of cirri and membranelles was exhibited by beating cilia and by paralyzed cilia regardless of whether the potential changes were spontaneous, evoked by mechanical stimulation, or evoked by the injected current.

In specimens with transected neuromotor fibrils, depolarizing current injected at one end of the cell evoked reversal of the organelles on both sides of the incision (Fig. 3). The reasons for Taylor's (*1*) conclusion that fibrillar transection abolishes coordination are not clear. However, we did find that massive incisions cause some deterioration and depress spontaneous fluctuations in membrane potential. By supplying a depolarizing current we have shown that the organelles still reverse in unison to depolarization after transection of the fibrils.

The evidence reported here and elsewhere (*5*, *11*) indicates that the coordination of ciliary orientation results primarily from electrotonic spread of spontaneous and evoked membrane potentials (Figs. 1A and 2) which control the positions of the individual cilia, cirri, and membranelles (Fig. 4). The view that systems of fibrils coordinate the orientation of cilia (*12*) is no longer credible.

YUTAKA NAITOH*
ROGER ECKERT
Department of Zoology and *Brain Research Institute, University of California, Los Angeles 90024*

References and Notes

1. C. V. Taylor, *Univ. Calif. Publ. Zool.* **19**, 403 (1920).
2. *Euplotes* propels itself either by creeping with its cirri (tapered organelles comprised of fused cilia) or by swimming with its membranelles (similar but more closely spaced organelles) and cirri. At irregular intervals the creeping or swimming organism spontaneously turns somewhat to the right or, in a more vigorous response, reverses rapidly and briefly before resuming forward locomotion to the right. The turning and reversal sequences are degrees of the familiar "avoiding reaction" originally described in *Paramecium* (*13*). The avoiding reaction of *Euplotes*, like that of *Paramecium*, is also induced by collision with an obstacle. The direction of locomotion depends on the orientation of movement exhibited by the cirri and membranelles. When directed posteriorly the active organelles propel the organism forward; when they reorient (reverse) completely so as to point anteriorly, they propel the organism backward (*1*).
3. R. Gliddon, *Progr. Protozool.* **91**, 246 (1965).
4. A. Okajima and H. Kinosita, *Comp. Biochem. Physiol.* **19**, 115 (1966).
5. Y. Naitoh and R. Eckert, *Science* **164**, 963 (1969).
6. H. Kinosita, S. Dryl, Y. Naitoh, *J. Fac. Sci. Univ. Tokyo Sec. IV Zool.* **10**, 303 (1964).
7. Y. Naitoh and R. Eckert, *Z. Vergl. Physiol.* **61**, 427 (1968).
8. L. Kuznicki, *Acta Protozool.* **1**, 301 (1963).
9. Y. Naitoh, *Science* **154**, 660 (1966).
10. T. Yamaguchi, *J. Fac. Sci. Univ. Tokyo Sec. IV Zool.* **8**, 573 (1960).
11. A. Okajima, *Jap. J. Zool.* **11**, 87 (1953); Y. Naitoh, *Annot. Zool. Jap.* **31**, 59 (1958).
12. J. A. Kitching, in *The Cell and the Organism*, J. A. Ramsay and V. B. Wigglesworth, Eds. (Cambridge Univ. Press, Cambridge 1961); M. A. Sleigh, *The Biology of Cilia and Flagella* (Macmillan, New York, 1962); A. D. Giese, *Cell Physiology* (Saunders, Philadelphia, ed. 2, 1962); T. H. Bullock and G. A. Horridge, *Structure and Function in the Nervous Systems of Invertebrates* (Freeman, San Francisco, 1965), vol. 1, p. 441; S. Ochs, *Elements of Neurophysiology* (Wiley, New York, 1965).
13. H. S. Jennings, *Amer. J. Physiol.* **2**, 311 (1899); *ibid.* **3**, 229 (1900).
14. Supported by PHS grant NB-8364 and NSF grant GB-7999.
* On leave of absence from the Zoological Institute, Faculty of Science, University of Tokyo, Tokyo, Japan.

14 August 1969; revised 7 October 1969

Codon Recognition by Enzymatically Mischarged Valine Transfer Ribonucleic Acid

Abstract. *Direct evidence for the adaptor hypothesis has been obtained by examining the codon recognition of a purified* Escherichia coli *valine transfer ribonucleic acid which was enzymatically mischarged with phenylalanine labeled with carbon-14 by reaction with purified phenylalanyl-transfer ribonucleic acid synthetase from* Neurospora crassa. *The mischarged transfer ribonucleic acid recognized the valine codons but failed to recognize the phenylalanine codon when tested in trinucleotide-directed ribosomal binding assay.*

The adaptor hypothesis of Crick (*1*) states that the amino acid sequence of a protein is determined, during the course of the translation and protein synthesis, by the alignment of aminoacyl transfer ribonucleic acids (tRNA) at corresponding nucleotide triplets (codons) in messenger RNA (mRNA). The specificity of the translation mechanisms depends on base pairing between a nucleotide region of tRNA, the anticodon, and nucleotides in the codon. The hypothesis predicts that the amino acid bound to tRNA does not participate in this recognition. The first evidence for the passive role of the amino acid was obtained by Chapeville *et al.* (*2*); they showed that [^{14}C]cysteine, while attached to its tRNA (tRNA^{Cys}), (*3*), was converted by catalytic reduction with Raney nickel to [^{14}C]alanine. When the resulting complex, [^{14}C]-alanyl-tRNA^{Cys}, was tested in a protein synthesizing system, radioactivity was incorporated under the direction of poly UG, a polymer which normally stimulates the incorporation of cysteine but not alanine (*2*). Similarly, von Ehrenstein *et al.* (*4*) found that the chemically altered aminoacyl-tRNA delivered [^{14}C]alanine, in place of cysteine, in a peptide of hemoglobin.

We used a different system to confirm the results of Chapeville *et al.* (*2*). Our approach was made possible by the discovery (*5*) that a phenylalanyl-tRNA synthetase of *Neurospora crassa* can mischarge phenylalanine onto two *Escherichia coli* tRNA's which have been identified as tRNA^{Val} and tRNA^{Ala} (*6*). Earlier studies had indicated that the phenylalanyl-tRNA^{Ala} responded to poly UCG in the same manner as alanyl-tRNA^{Ala} of *N.* crassa and *E. coli* (*6*, *7*). We have asked whether va-

Table 1. The incubation mixture (0.05 ml) contained 0.05*M* tris-acetate (*p*H 7.2), 0.05*M* KCl, 0.03*M* magnesium acetate, and 2.1 O.D. units (260 nm) of *E. coli* ribosomes. Trinucleotides [0.1 O.D. units (260 nm)] and [^{14}C]aminoacyl-tRNA's were added as indicated. Incubation was carried out at 24°C for 20 minutes. The reaction mixture was diluted with 3 ml of buffer and filtered through Millipore filters; the radioactivity was measured in a Tricarb liquid scintillation counter.

Trinucleotides	[^{14}C]Valyl-tRNA^{Val} (5.5 pmole)		[^{14}C]Phenylalanyl-tRNA^{Val} (4.8 pmole)		[^{14}C]Phenylalanyl-tRNA (7.4 pmole)	
	pmole	Δpmole	pmole	Δpmole	pmole	Δpmole
None	0.298		0.301		0.223	
GUC	0.300	0.002	0.298	−0.003		
GUU	0.511	0.213	0.598	0.297		
GUA	1.005	0.707	1.203	0.902		
GUG	1.259	0.961	1.315	1.014		
UUU	0.287	−0.011	0.291	−0.010	0.516	0.293

line or phenylalanine codons are recognized when the mischarged tRNA is tested in a codon-directed ribosome binding assay.

We used *E. coli* tRNA^{Val} (acceptance of 1100 pmole/O.D. unit, measured at 260 nm) purified by reversed-phase chromatography (*8*). *Neurospora crassa* phenylalanyl-tRNA synthetase (peak C), one of three chromatographically separable, cytoplasmic phenylalanyl-tRNA synthetases, was purified as described by Kull and Jacobson (*9*). The aminoacylation of *E. coli* tRNA^{Val} by *N. crassa* phenylalanyl-tRNA synthetase was performed according to Ritter *et al.* (*10*). The reaction system contained (in 1 ml): 50 m*M* potassium-cacodylate buffer, *p*H 6.3; 0.5 μmole of ATP; 7.5 μmole of $MgCl_2$; 2.7 nmole of [^{14}C]-phenylalanine [817 disintegrations per minute (dpm)/pmole; and 70 pmole of *E. coli* tRNA^{Val}. Aminoacylation of *E. coli* tRNA^{Val} with [^{14}C]valine and of unfractionated *E. coli* tRNA with [^{14}C]-phenylalanine were performed with a crude mixture of *E. coli* aminoacyl-tRNA synthetases (*11*). *Escherichia coli* ribosomes were prepared from *E. coli* B (General Biochemicals) as described (*12*). The trinucleotides GUU, GUC, GUA, and GUG were prepared enzymatically with ribonuclease T_1 from guanosine 2′,3′-cyclic phosphate and appropriate dinucleotide phosphates (*13*). (UUU was a product of Miles Laboratories.) The binding of aminoacyl-tRNA to ribosomes was assayed by the procedure of Nirenberg and Leder (*14*).

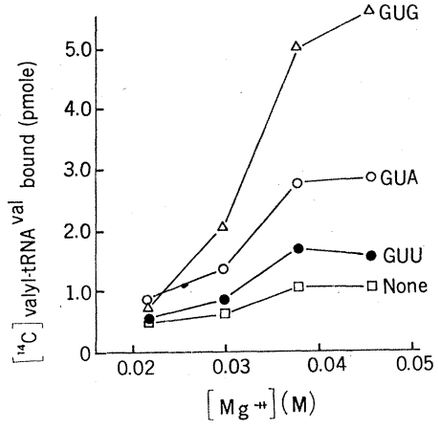

Fig. 1. Effect of Mg^{2+} concentration on the binding of [^{14}C]valyl-tRNA^{Val} to ribosomes in the presence of trinucleotides. The incubation mixture contained 27.7 pmole of [^{14}C]valyl-tRNA^{Val}, 0.1 O.D. unit (260 nm) of the indicated trinucleotides, and the specific concentration of magnesium acetate. The remaining conditions were as described in Table 1.

Since the codon assignments (*15*) for valine are GUU, GUC, GUA, and GUG, and for phenylalanine are UUU and UUC, we tested the binding of [^{14}C]valyl-tRNA^{Val} and [^{14}C]phenylalanyl-tRNA^{Val} to ribosomes in the presence of these triplets. Both [^{14}C]valyl-tRNA^{Val} and [^{14}C]phenylalanyl-tRNA^{Val} recognized GUG and GUA and to a lesser extent GUU (Table 1). The binding of the [^{14}C]phenylalanyl-tRNA^{Val} to these valine codons was quantitatively similar to that obtained with [^{14}C]valyl-tRNA^{Val}. At the same time, *E. coli* tRNA^{Val}, whether charged with valine or phenylalanine, failed to recognize the phenylalanine codon UUU. In contrast, the [^{14}C]phenylalanyl-tRNA, synthesized in a homologous system containing both *E. coli* aminoacyl-tRNA synthetase and unfractionated *E. coli* tRNA, gave the expected positive response to the UUU codon (Table 1, last column). These codon response data are consistent with studies of Jacobson (*16*) demonstrating that phenylalanyl-tRNA^{Val} and phenylalanyl-tRNA^{Ala} do not allow phenylalanine to enter into normal positions of the hemoglobin chain when tested in a subcellular reticulocyte system. Other results indicated that [^{14}C]phenylalanyl-tRNA$^{Val+Ala}$ failed to support the incorporation of phenylalanine into protein in the presence of poly U and ribosomes (*17*).

The response of a highly purified *E. coli* valyl-tRNA^{Val} to the codons GUG, GUA, and GUU is of interest because this pattern of codon recognition is not predicated in the wobble hypothesis of Crick (*18*). Similar results, however, have been obtained in studies with the major *E. coli* valine tRNA (*19, 20*), a yeast valine tRNA (*21*), and a rat liver valine tRNA (*22*). Since the binding assay is strongly dependent on Mg^{2+}, we examined the effect of Mg^{2+} concentration on the ability of *E. coli* valyl-tRNA^{Val} to recognize these codons (Fig. 1). At a Mg^{2+} concentration of 0.02*M*, significant stimulation of binding occurred with GUG and GUA, but not with GUU. The stimulatory effect of GUU became apparent only at higher Mg^{2+} concentrations. These results could explain a possible discrepancy with the data of Yaniv and Barrell (*23*), who used 0.02*M* Mg^{2+} in their binding assays (*24*). However, Nishimura (*17*) found Val-tRNA^{Val} bound to the GUU-ribosome complex at 0.02*M* Mg^{2+}. Although the primary sequence of *E. coli* tRNA^{Val} has been elucidated (*23*), the nucleotide in the 5′-position of the anticodon region has not been fully characterized. Studies suggest that it is a uridine derivative with an additional residue, presumably a carboxyl group (*23, 25*). Its specific function may be recognition of both A and G, and to a lesser degree U, in the third position of valine codons, not only in *E. coli* (*19*), but perhaps also in a valine tRNA of yeast (*21*) and rat liver (*22*).

These results provide direct evidence that an amino acid, once attached to tRNA, does not itself participate in codon recognition. The decisive role in codon recognition is played by the precise structure of the tRNA.

DEZIDER GRUNBERGER
I. BERNARD WEINSTEIN
Institute of Cancer Research and
Department of Medicine, Columbia University College of Physicians and Surgeons, New York 10032
K. BRUCE JACOBSON
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

References and Notes

1. F. H. C. Crick, *Symp. Soc. Exp. Biol.* **12**, 138 (1958).
2. F. Chapeville, F. Lipmann, G. von Ehrenstein, B. Weisblum, W. J. Ray, Jr., L. B. Benzer, *Proc. Nat. Acad. Sci. U.S.* **48**, 1086 (1962).

3. Abbreviations are: A, adenosine; C, cytidine; G, guanosine; U, uridine; cys, cysteine; ala, alanine; val valine; poly U, poly uridylic acid; poly UG, poly uridylic-guanylic acid; O.D.,

optical density: and tris, tris(hydroxymethyl)-aminomethane.
4. G. von Ehrenstein, B. Weisblum, S. Benzer, *Proc. Nat. Acad. Sci. U.S.* **49**, 669 (1963).
5. W. E. Barnett and K. B. Jacobson, *ibid.* **51**, 642 (1964).
6. W. E. Barnett and J. L. Epler, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 549 (1966).
7. J. L. Epler, W. E. Barnett, K. B. Jacobson, *Fed. Proc.* **24**, 408 (1965).
8. J. F. Weiss, R. L. Pearson, A. D. Kelmers, *Biochem. J.* **7**, 3479 (1968).
9. F. J. Kull and K. B. Jacobson, *Proc. Nat. Acad. Sci. U.S.* **62**, 1137 (1969).
10. P. O. Ritter, F. J. Kull, K. B. Jacobson, *Biochim. Biophys. Acta* **179**, 524 (1969).
11. V. Z. Holten and K. B. Jacobson, *Arch. Biochem. Biophys.* **129**, 283 (1969).
12. D. Grunberger, L. Meissner, A. Holý, F. Šorm, *Collect. Czech. Chem. Commun.* **32**, 2625 (1967).
13. D. Grunberger, A. Holý, F. Šorm, *ibid.* **33**, 286 (1968).
14. M. Nirenberg and P. Leder, *Science* **145**, 1399 (1964).
15. —→–, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, C. O'Neal, *Proc. Nat. Acad. Sci. U.S.* **53**, 1161 (1965).
16. K. B. Jacobson, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 719 (1966).
17. S. Nishimura, personal communication.
18. F. H. C. Crick, *J. Mol. Biol.* **19**, 548 (1966).
19. D. A. Kellogg, B. P. Doctor, J. E. Loebel, M. W. Nirenberg, *Proc. Nat. Acad. Sci. U.S.* **55**, 912 (1966).
20. K. Oda, F. Kimura, F. Harada, S. Nishimura, *Biochim. Biophys. Acta* **179**, 97 (1969).
21. A. D. Mirzabekov, D. Grunberger, A. I. Krutilina, A. Holý, A. A. Bayev, F. Šorm, *ibid.* **176**, 75 (1968).
22. S. Nishimura and I. B. Weinstein, *Biochemistry* **8**, 832 (1969).
23. M. Yaniv and B. G. Barrell, *Nature* **222**, 278 (1969).
24. M. Yaniv, personal communication.
25. F. Harada, F. Kimura, S. Nishimura, *Biochim. Biophys. Acta* **182**, 590 (1969).
26. Supported by NIH grant R10 CA-02332, and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation. I.B.W. is a Career Scientist of the Health Research Council of the City of New York (I-190). We thank Dr. J. F. Weiss for the *E. coli* $tRNA^{Val}$ and Miss G. Carvajal for technical assistance.

25 August 1969

Microtubular Protein: Synthesis and Metabolism in Developing Brain

Abstract. *In the developing mouse brain 40 percent of the labeled soluble protein found after injection of leucine-C^{14} consists of subunits with a molecular weight of 60,000 and with other characteristics of microtubular protein. This protein has a half-life of about 4 days.*

Microtubular protein binds colchicine (*1, 2*), is composed of subunits with a molecular weight of 60,000 (*3*), and is precipitable by vinblastine (*4, 5*). Brain and axoplasm contain significant amounts of this protein (*1, 3*), and there is evidence that microtubules participate in the rapid transport of proteins from the nerve cell body down the axon (*6*).

Because of the abundance of this protein in brain and its probable importance in axoplasmic transport (*7*), we have studied its synthesis and metabolism in the brains of developing and adult mice.

Swiss albino mice, 1 or more days of age, were injected intracerebrally with 2 μc of L-leucine-1-C^{14} [30 mc/mmole, New England Nuclear (*8*)]. After injection, the mice were decapitated, and the brains were removed and homogenized in 0.01*M* phosphate buffer, *p*H 6.5, containing 0.01*M* $MgCl_2$ and 0.24*M* sucrose. Soluble and particulate components were separated by centrifugation for 1 hour at 100,000*g*.

For determination of colchicine binding activity (*3*), 1 to 2 mg of protein were incubated with 1 μc of colchicine-H^3 (2 c/mmole, New England Nuclear) and the mixture was filtered through DEAE-cellulose–impregnated filter paper (No. DE81, Whatman). For estimation of the molecular weight, the proteins were disaggregated in 0.01*M* phosphate buffer (*p*H 6) containing 0.1 percent sodium dodecyl sulfate and 0.5*M* urea, reduced with 0.15*M* β-mercaptoethanol, alkylated with 0.02*M* iodacetamide, concentrated, and subjected to electrophoresis in 0.01*M* phosphate buffer, 0.1 percent sodium dodecyl sulfate (*p*H 6) (*9, 10*). Migration in this system is proportional to molecular weight (*11*).

Results of the above-mentioned procedures were as follows. Approximately 40 percent of the labeled protein in the 100,000*g* supernatant from homogenized brains of 2- or 6-day-old mice which had received the intracerebral injection of leucine-1-C^{14} the day before was present as a single peak with a molecular weight of approximately 60,000 (Fig. 1). One milligram of the supernatant protein bound about 72,000 counts of colchicine-H^3 per minute. Upon the addition of vinblastine ($10^{-3}M$) to the supernatant a visible precipitate formed (*4*). This precipitate contained approximately 35 percent of the total soluble protein and 98 percent of the colchicine bound to protein. Electron micrographs of negatively stained preparations of the precipitate showed the characteristic morphology of microtubular crystals (*5, 12*). Gel electrophoresis showed that the precipitate contained the protein peak with molecular weight of 60,000, whereas most of the other labeled protein remained in the supernatant after addition of vinblastine (Fig. 1). After electrophoresis of the protein precipitated with vinblastine, addition of Coomassie blue revealed a deeply stained band of molecular weight 60,000; also, three faint, closely spaced, narrow bands of very high molecular weight were detected; these were not substantially labeled with leucine-C^{14}. When the initial vinblastine precipitate was dissolved by dialysis against phosphate buffer and precipitated again with vinblastine and magnesium, these contaminants were no longer detectable. The purified precipitate contained about 20 percent of the protein in the initial 100,000*g* supernatant and about 30 percent of the total incorporated leucine-C^{14}.

The microtubular protein represented 30 to 40 percent of the labeled soluble protein in mice injected with

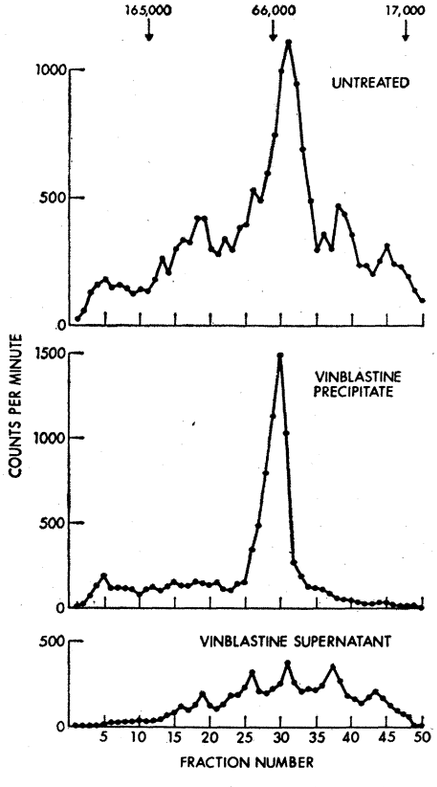

Fig. 1. Gel electrophoresis of protein in supernatant (100,000*g*) obtained from brain homogenates 24 hours after injection of leucine-C^{14}. Electrophoresis was conducted for 9 hours at 80 volts on polyacrylamide gels. The untreated supernatant (top), the protein precipitated by vinblastine (middle), and supernatant protein remaining after vinblastine precipitation (bottom) were subjected to electrophoresis simultaneously. Markers for the molecular weight estimate were a mixture of nonreduced human γ-globulin (165,000), bovine serum albumin (66,000), and hemoglobin monomers (17,000). These were subjected to electrophoresis simultaneously in a separate gel and localized by staining with Coomassie blue.