of β_2M globulin, it is possible that the antibody is present at birth but because of the low concentration of the β_2M is not detected by our methods (*8*).

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Coding Ambiguity in Cell-Free Extracts of Chlamydomonas

Abstract. *A cell-free system that incorporates amino acids into polypeptide has been prepared from extracts of the alga* Chlamydomonas. *The addition of polyuridylic acid to this system stimulates the incorporation both of phenylalanine and of leucine.*

The discovery that polyuridylic acid (*1*) and other synthetic polyribonucleotides can direct the incorporation of amino acids into polypeptides in cell-free extracts of *Escherichia coli* (*2*) has provided a valuable tool for studies of the genetic code (*3*) as well as of the role of ribosomes in protein synthesis (*4*). Synthetic polyribonucleotides can also direct the incorporation of amino acids in cell-free extracts of animal cells (*5*).

We have prepared a cell-free system from extracts of exponentially growing cultures of *Chlamydomonas* which can incorporate amino acids into polypeptides. *Chlamydomonas* is a unicellular sexual microorganism, an alga, that combines the subcellular organization of cells of higher plants and animals (*6*) with the exponential growth of microbial populations. As a phytoflagellate in the group from which both the higher plants and the animals have evolved (*7*), *Chlamydomonas* is in a central phylogenetic position with respect to considerations of the universality of the genetic code. The organism is being used for studies of nucleic acid and protein synthesis, especially for comparison with bacterial and mammalian cells (*8*).

The organism (strain 21gr) was grown on a minimal culture medium (*8*). Cultures were grown from log-phase inocula for four to eight generations with a constant 6-hour doubling time. Under these conditions, the concentrations of RNA and protein per cell remain constant until the culture reaches a density of about 5×10^6 cells per milliliter (*8*). In these experiments, cells were harvested when growth reached 2 to 3×10^6 cells per milliliter, washed with standard buffer (tris-HCl, *p*H 7.8, $10^{-2}M$; magnesium acetate, $10^{-2}M$; KCl, $6 \times 10^{-2}M$; mercaptoethanol, $6 \times 10^{-3}M$) (*2*), and resuspended in 5-ml total volume per gram, wet weight, of cells. The harvesting and all subsequent preparative steps were carried out at 2°C. The cells were broken with a Mullard ultrasonic oscillator (10 kv) for 3 minutes (95 to 99 percent breakage). The broken-cell suspension was centrifuged at 10,000*g* for 10 minutes and the supernatant fluid was recentrifuged at 30,000*g* for 30 minutes. The resulting supernatant fraction (S-30) was separated into a ribosomal and supernatant fraction (S-100) by centrifugation for 2 hours at 105,000*g*. The pellet was suspended in standard buffer that also contained 0.1 percent DOC and was resedimented at 105,000*g* for 2 hours. The supernatant fluid was discarded, the tube was rinsed with 2 ml of buffer, and the pellet was resuspended in one-tenth of the original volume of the same buffer. Soluble RNA was prepared by phenol extraction (*5*) of the S-100 fraction. The S-30, S-100, and DOC ribosome fractions were prepared on the same day on which they were used for incorporation studies. The reagents used, as well as the procedures for incubation, precipitation, and radioactive counting were those of Nirenberg and Matthei (*2*) as modified by Weinstein and Schechter (*5*). The composition of the reaction mixture is given in Table 1.

The extent of incorporation of C^{14}-phenylalanine into polypeptide by cell-free extracts of *Chlamydomonas* is shown in Table 1. The S-30 fraction had a low endogenous activity, but the addition of 100 μg of polyU to this system resulted in a 31-fold stimulation of phenylalanine incorporation. Supplementing the system with sRNA from *Chlamydomonas* increased this incorporation from 31-fold to about 61-fold. A comparable enhancement of the polyU stimulation was also obtained

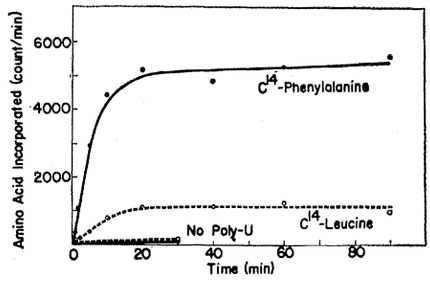

Fig. 1. Rate of polyU-stimulated phenylalanine and leucine incorporation in the *Chlamydomonas* system. The reaction mixture is as described in Table 3. Reactions were stopped at the indicated times by addition of TCA.

Table 1. Polyuridylic acid stimulation of C^{14}-phenylalanine incorporation by cell-free fractions of *Chlamydomonas*. When polyU was present in the reaction it was added in 100 μg amounts. The reaction mixture contained the following (in micromoles unless otherwise specified): tris-HCl buffer, *p*H 7.8, 2.3; magnesium acetate, 2.3; KCl 13.8; mercaptoethanol, 1.3; ATP, 0.25; PEP, 1.25; GTP, 0.01; CTP, 0.01; UTP, 0.01; PEP kinase, 12 μg; C^{14}-phenylalanine, 0.8 (160 mc/mole); and a mixture of 19 C^{12} amino acids excluding phenylalanine, 0.0125 each. The other components are specified in the table. The total volume of the reaction mixture was 0.3 ml. Tubes were incubated 40 min at 35°C. The reaction was stopped with TCA. The product was prepared for counting (*5*). Contents of ribosomal protein in the fractions were 87 μg in the S-30 and 62 μg in the ribosomes. The S-100 contained 0.31 μg protein and was prepared by two successive centrifugations of the supernatant at 105,000*g* for 2 hours to insure against contamination by ribosomes.

Cell fraction	Additions	C^{14}-phenylalanine incorporated	
		Radioactivity (count/min)	Ribosomal protein (mμmole/mg)
S-30	None	71	0.15
S-30	PolyU	2239	4.7
S-30	PolyU; 42 μg sRNA *	4222	8.9
Ribosomes	PolyU	0	0
S-100	PolyU	0	0
Ribosomes plus S-100	PolyU; 42 μg sRNA *	1346	4.0

* From *Chlamydomonas*.

with sRNA from *Escherichia coli*. Further fractionation of the S-30 indicated that both the S-100 and the ribosome fractions were required for activity. The polyU-directed incorporation of phenylalanine in the *Chlamydomonas* system was in the range of 9 mμmole/mg of ribosomal protein, as compared with the value of 20 obtained with *E. coli* extracts (*3*).

The general requirements of the *Chlamydomonas* system, as well as the effects of various inhibitors, are similar to those previously reported for extracts of *E. coli* (*2*) and animal cells (*5*) (Table 2). The dependence upon added ATP and an ATP-generating system indicates that the incorporating activity does not result from whole-cell contamination, unlikely in any event considering the size of the organism (5 to 8 μ) and its fragility. Only partial inhibition of activity was observed with puromycin and chloramphenicol. Incubation of the S-30 fraction for 10 minutes at 37°C in a complete system, before addition of polyU, resulted in a loss of 46 percent of the radioactivity observed in the absence of such incubation. Similar incubation in the presence of deoxyribonuclease (15 μg/ml) gave comparable results, whereas the presence of ribonuclease (15 μg/ml) caused 100-percent inhibition.

The specificity of polyU was shown by the fact that polycytidylic, polyadenylic, and polyinosinic acids, either with or without added sRNA, failed to stimulate the incorporation of phenylalanine. In the same experiment addition of polyU led to a 38-fold stimulation of phenylalanine incorporation, without added sRNA, and a 71-fold stimulation with supplementary sRNA from *Chlamydomonas*.

Sensitivity of the reaction to magnesium was tested when the Mg concentration was from 5×10^{-3} to $5 \times 10^{-2}M$; the optimum concentration for incorporation both with and without polyU was in the region of $1 \times 10^{-2}M$. Studies of *Chlamydomonas* ribosomes (*8*) in the ultracentrifuge have shown that at $1 \times 10^{-2}M$ Mg they sediment at 70*S*, whereas at $1 \times 10^{-3}M$ Mg they are dissociated into a mixture of 50*S* and 30*S* particles that can be reaggregated by restoring the magnesium concentration to $1 \times 10^{-2}M$. These results are similar to those on the magnesium sensitivity of *E. coli* ribosomes (*9*).

The optimal concentration for the polyU was 100 μg/0.3 ml (the volume of the reaction mixture) with and without added sRNA, and with several different S-30 preparations. The optimal concentration for added sRNA varied somewhat with different preparations but was in the range of 50 μg/0.3 ml.

In addition to phenylalanine 14 amino acids labeled with C^{14} were tested individually for their incorporation into polypeptide in the absence and presence of polyU (Table 3). Each of the amino acids was incorporated by the endogenous system. Polyuridylic acid caused a stimulation of leucine incorporation 17 percent of that of phenylalanine. This was verified with a preparation containing less than 0.1 percent nonuridylic acid residues (*10*). No apparent stimulation was noted with the other amino acids.

The kinetics of polyU-stimulated incorporation of leucine and phenylalanine into polypeptide were compared, as shown in Fig. 1. Both reactions started with no apparent lag and proceeded at a linear rate for approximately 10 minutes. By 20 minutes, the incorporation of both amino acids reached a plateau, where the incorporation of leucine was about 22 percent of that of phenylalanine.

The kinetic studies suggest that polyU stimulation of the incorporation of leucine does not require conversion of polyU to a polymer containing other nucleotide residues. This inference is supported by the fact that polyU did not stimulate the incorporation of amino acids other than leucine and phenylalanine.

In unpublished studies we have found that the addition of polyuridylic-cytidylic acid to the *Chlamydomonas* system stimulates the incorporation of leucine considerably more than would be expected if only triplets containing two uridylic and one cytodylic acid residue were coding for leucine. These results, too, suggest that coding units consisting entirely of uridylic acid residues can direct the incorporation of leucine in the *Chlamydomonas* system.

That leucine and phenylalanine both respond to a sequence of uridylic acid residues indicates that coding ambiguity, that is, the response of two amino acids to the same nucleotide sequence, does occur in the *Chlamydomonas* cell-free system. A stimulation of leucine incorporation by polyU has also been reported in studies with extracts of *E. coli* (*3, 11*). It remains to be determined whether ambiguity also occurs in vivo, where an additional mechanism would be required to avoid confusion in incorporation of leucine and phenylalanine into protein.

Studies with polyUC, polyUA, and polyUG in the *Chlamydomonas* system have indicated no qualitative differences from the published data on *E. coli* for the amino acids phenylalanine, leucine, serine, isoleucine, tyrosine, and

Table 2. Requirements and inhibitors of polyU-stimulated incorporation of C^{14}-phenylalanine by the S-30 fraction of *Chlamydomonas*. The reaction mixture is like that of Table 1. Components added or omitted are specified in the table. PolyU concentration was 100 μg per tube.

Tube No.	Additions	Phenylalanine incorporated (count/min)	Inhibition (%)*
	S-30 fraction		
1	PolyU	3175	0
2	PolyU; omitted: ATP, PEP, PEP kinase	25	99
3	PolyU, puromycin (5 μg)	1751	45
4	PolyU, 32 μg chloramphenicol	2249	24
	S-30 incubated 37°C 10 min before the indicated additions		
5	PolyU	1703	46
	S-30, incubated 37°C, 10 min, with 5 μg DNAase before the indicated additions		
6	PolyU	1578	7
	S-30 incubated 37°C, with 5 μg RNAase before the indicated additions		
7	PolyU	0	100

* The percentage inhibition in tubes 2, 3, and 4 was calculated on the basis of that observed with 1, whereas in tubes 6 and 7 it was calculated for that observed with 5.

Table 3. Incorporation into polypeptide of 15 amino acids by the S-30 fraction from *Chlamydomonas*.

Amino acid	Net counts per min		Phenylalanine stimulation (%)
	No polyU	100 μg polyU	
Phenylalanine	290	1250	100
Leucine	145	312	17.4
Isoleucine	44	67	2.4
Tyrosine	310	262	0
Valine	54	74	2.1
Proline	37	52	1.6
Serine	52	38	0
Alanine	76	60	0
Glycine	32	23	0
Arginine	27	24	0
Lysine	54	50	0
Aspartic acid	31	22	0
Glutamic acid	37	30	0
Threonine	23	34	1.1
Histidine	119	96	0

The reaction mixture is in Table 1. A mixture of 19 C^{12}-L-amino acids, excluding the C^{14}-amino acid being tested, was added, 0.0125 μmole each. The C^{14}-amino acids under test contained 850 to 1000 μc/mg radioactivity (*5*) and were each added at 0.8 mμmole. The S-30 fraction contained 0.19 mg of ribosomal protein; 60 μg of SRNA was added.

valine (*12*). These studies add to the accumulating evidence that the genetic code is, at least in its general features, the same in widely divergent species (*13*).

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References and Notes

1. The abbreviations are polyU, polyuridylic acid; PEP, phosphoenolpyruvate; ATP, GTP, CTP, UTP, adenosine, guanosine, cytidine, and uridine triphosphates; DOC, deoxycholate; sRNA, soluble RNA; TCA, trichloroacetic acid; polyUC, polyuridylic-cytidylic acid; polyUA, polyuridylic-adenylic acid; polyUG, polyuridylic-guanylic acid.
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Prolonged Immobilization of the Body: Changes in Performance and in the Electroencephalogram

Abstract. *Subjects who were immobilized for a week but otherwise were exposed to a normal and varied sensory environment showed intellectual and perceptual deficits similar in many respects to those occurring after prolonged visual and auditory deprivation. A significant change in the electroencephalogram was also observed.*

Severe deprivation of vision and hearing, in an isolation chamber, for periods up to a week can produce widespread impairments of intellectual and perceptual processes. Hallucinatory phenomena, to a limited degree, may also be present (*1–3*). Furthermore, these behavioral deficits are accompanied by significant changes in the electrical activity of the brain (*4–6*). The purpose of this experiment was to determine whether similar effects can be produced in subjects whose tactile-kinesthetic activity is reduced via immobilization but who otherwise are exposed to a normal and varied sensory environment. Fiske, in a recent review of the literature on perceptual deprivation, suggested that such results may be possible (*1*). Unfortunately, behavioral experiments specifically designed to appraise the role of kinesthesis are rare. Most of the research in this area has usually employed various degrees of immobility of the body concurrent with restriction of visual and auditory stimulation (*1, 7*). The results, therefore, are inconclusive. In the present study, tactile-kinesthetic stimulation alone was reduced.

The subjects were 22 male university students who were placed in a "coffin-like" box for 1 week—a period similar to the one we used in earlier experiments on visual and auditory deprivation (*2, 3*). This box was 7 feet long, 28 inches wide and 18 inches high. It was lined with a thick layer of foam rubber cut out in the shape of a human figure. At one end of the box was located an adjustable, padded head-holding device which fitted snugly against the forehead and side of the head. No padding was placed over the ear section. A plastic frame, to which pictures could be attached, was placed some distance above the head-holder. At the other end of the box, two V-shaped restraining devices immobilized the feet.

The subject was told to lie on the foam rubber with his head in the padded holder and his feet in the restraining holders. His legs and trunk were immobilized by means of belts fastened to the base of the box. The belts did not interfere with circulation. The arms were placed in comfortable but rigid cylinders which were fastened down but which permitted some degree of flexion of the elbow. To minimize such factors as pain and cramps the subjects were unstrapped periodically—for example, for 15 minutes at mealtimes and for 1 hour in the afternoon. During this hour they went to the washroom and also took a battery of intellectual tests. They were also unstrapped for 9 hours during the night but were not allowed to sit up or stand up. Apart from these restrictions on motor activity, they were exposed to as normal an environment as possible. They could hear people moving about and talking. They could listen to a radio whenever they wished. Various pictures were placed above them and changed from time to time, and the lights were put out at night. An experimenter was on duty at all times.

The subjects were given two batteries of tests, intellectual and perceptual-motor. These were identical with those used in our earlier experiments (*2, 3*). The intellectual tests, measuring 12 different abilities, were administered before the experiment and at daily intervals during the week. Eight equivalent forms of each test were used. Some of the tests measured fairly simple abilities involving overlearned material, while others appraised more complex abilities requiring deliberation and manipulation of ideas. The tests were all short, of several minutes' duration each, and consisted of the following: simple arithmetic problems, mathematical reasoning (solving numerical sequences), abstract reasoning (solving sequences of patterns), verbal fluency (writing words beginning with a certain letter), verbal reasoning (for example, *lend* is to *borrow* as *rich* is to ——), space visualization (selecting the exact parts which will form a certain design), digit span (forward and backward), rote learning of a list of nine three-letter words, recall of words presented a few minutes earlier, recognition of words presented earlier, cancellation test (picking out a particular number from pages of randomized numbers), and dexterity (placing a dot in a small triangle, making two check marks in a square, and tracing a line through a maze without touching the sides).

The battery of five perceptual-motor tests was only given before and after the week of immobilization. It consisted of the following: (i) Depth