impart a clear red color to the vertebral stripe. Albinos with xanthophores appear green when placed on a dark background in reflected light. Other albinos with neither allophores nor xanthophores may be 'brown' albinos. The guanophores are not continuous across the back of the frog. A narrow band around each dorsal stripe where the chromatophores are absent accounts for the conspicuous light area surrounding the head and body patterns.

Live tree frogs were collected from more than 100 localities from British Columbia to Baja California during the years 1961 to 1963. The colors of the frogs from some of these localities are listed in Table 1. The frequency of red frogs is greatest in xeric areas; the hue and intensity of the red color ranges from a bronze in the frogs from Lake Arrowhead and Oregon to a more 'iron-rust' red in frogs from San Diego and Baja California.

Pyburn (*2*) found that the red color of the vertebral stripe of the cricket frog (*Acris crepitans*) is controlled by a single dominant gene. Other crosses led Pyburn to conclude that green is also under the control of a dominant allele of a single gene. Our data suggest that green color in *Hyla regilla* is determined by genes from at least two different loci. After the breeding season, green cricket frogs "fade" to grey and in the spring become green once more. Pacific tree frogs lighten and darken in response to hormones, light, and temperature, but the colors retain their structural integrity. A different structural and metabolic basis for the colors in these two genera is suggested.

Green frogs vary in hue and intensity from a dark, almost black, green (Sierra Mountains) to grass-green individuals (Southern California and Baja California) to blue frogs (Southern Oregon). The blue animals were collected (along with animals with red, brown, and green colors) in 1954 and again in 1961 in the same pond, which suggests that this blue is under genetic control. The blue color appears to be due to the Tyndall effect in the absence of xanthophores. The brown frogs range in color from yellow-tan to almost black. Preliminary examination of the 1962 crosses suggests that brown is controlled by at least one dominant gene; the variability suggests a much more complex system and more work is required.

Close examination of the frogs thus reveals that there is great variability within each color phase. The large number of different shades of green could be partially explained on the basis of the different alleles at the two loci involved in determining green color—that is, different alleles controlling the amount of guanine or carotenoid pigment produced. There may also be an interaction of one or a group of modifying genes, as was suggested by Volpe and Dasgupta (*3*) in the Burnsi complex in *Rana pipiens*.

LINDA E. RESNICK
DAVID L. JAMESON
Department of Biology,
San Diego State College,
San Diego, California

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Rationale for a Universal Genetic Code

Abstract. *A mutation in the genetic code would place new amino acids in certain loci and entirely eliminate amino acids from other loci of practically all proteins in an organism. It is reasonable to postulate that mutations of this kind cannot supplant the original code. The genetic code, once established, would therefore remain invariant.*

During the past decade a considerable body of evidence has accumulated in support of the hypothesis that a sequence of the nucleotide triplets in the DNA of an organism bears a one-to-one correspondence to the sequence of amino acids in the proteins synthesized by that organism. This implies that, given the nucleotide sequence in a strand of DNA, the amino acid sequence in the protein corresponding to that strand could be predicted. The abstract set of rules which associates a nucleotide triplet with a given amino acid is known as the genetic code. The genetic code is expressed in the organism as a set of molecular interactions. The molecules taking part in these interactions are believed to be DNA, messenger RNA (mRNA), a low molecular weight amino acid adaptor RNA (aRNA, also called acceptor, transfer, or soluble RNA), and an amino acid activating enzyme (aa-enzyme).

The following relationship is now believed to exist among these molecular species (*1*): The mRNA is synthesized on DNA and is complementary to it, so that, in principle, if the sequence of mRNA nucleotides is known, the sequence of nucleotides in the DNA can be deduced, and vice versa. In the process of protein synthesis the different amino acids are ordered in the protein molecule according to the sequence of mRNA nucleotide triplets. Each triplet will be called a codon, a term coined by Crick. Each amino acid is matched to its codon by amino acid–specific aRNAs. The structure of aRNA is still incompletely understood but it can be visualized as a short double-stranded molecule that can carry an activated amino acid with a stereospecific configuration of nucleotides at one end which we will designate by the letter C (*2*) and which will only form a bond with one particular codon. There should be as many different adaptor molecules in an organism as there are different codons. The amino acid is attached to the adaptor molecule by an amino acid activating enzyme (*1*). This enzyme should have two sites, A and R. The amino acid attaches to site A while site E of a particular adaptor RNA molecule attaches to R (Fig. 1). Sites A and R, and E and C are essentially independent (*3*).

It is safe to say that the synthesis of both the aa-enzyme and the aRNA molecules are under genetic control. For the aa-enzyme this assumption can be justified because there is no evidence, nor known biochemical mechanism, for the self-replication of protein molecules. Independent self-replication of adaptor RNA can be ruled out on theoretical grounds: If these molecules were independent, then as errors in replication occurred a heterogeneous population of adaptor molecules would result which would completely destroy the precision of the protein synthesis mechanism, a precision known to exist on the basis of analysis of protein structure.

Since DNA can mutate, the structures of the aa-enzyme and of the aRNA are mutable. Since the operation

of the genetic code is a function of the structure of these molecular entities, the genetic code, in principle, can mutate. The question is, can these mutations become established in the phylogenetic development of organisms?

This question may be answered by considering the consequences of mutations of the genetic code in a *haploid* organism at sites A, R, E, or C. We will first assume that only one mutation arises at a time. The symbols X and Y are taken to be two loci in any wild-type protein molecule at which amino acids A_x and A_y are respectively located.

Consider first the case where aa-enzyme site A, at which the amino acid A_x is attached, is genetically altered. This can lead to two classes of change in protein synthesis: (i) The specificity for amino acid A_x may be diminished and A_y will frequently or even always be attached to the A_x aRNA. This results in locus X sometimes or always being filled by the A_y amino acid. (ii) The aa-enzyme activity may decrease or cease entirely, resulting in locus X being filled inefficiently or not at all. Only changes of the first class could lead to a new code, but in both situations the amino acid composition of every newly synthesized protein in the organism can be altered in a random manner. An analogous situation would be a typewriter that randomly inserts certain letters incorrectly or omits them entirely. As Table 1 shows, changes in site E of the aRNA will have the same effect as changes in site A of the aa-enzyme. If site R of the enzyme is mutated the effect is somewhat different. Again there are two classes of results. (i) The enzyme may sometimes, or always, attach amino acid A_x to the aRN associated with A_y and therefore locus Y in all proteins will contain either A_x or A_y in random distribution. Locus X will be filled less efficiently or not at all. (ii) The aa-enzyme activity decreases, or ceases entirely. As before, this would not alter the code. Finally, there is site C on the aRNA. Unlike the previous sites only a limited number of changes are permitted, all tending to be expressed as all-or-none effects. If all possible codons exist and if site C mutates to match the codon for A_y, then A_x amino acids will be placed randomly in locus Y as will A_y amino acids. Locus X will not be filled.

Brody and Yanofsky (*4*) have postulated that certain suppressor mutations may be one of these types. Their mutants probably do not contain alterations in site C, since only a small percentage of particular amino acid loci are altered.

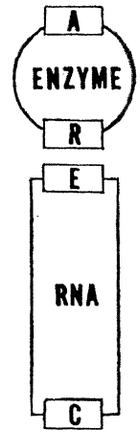

Fig. 1. Diagram of the activating enzyme adaptor RNA complex.

Changes in sites A, R, and E will produce essentially the same results in diploid or polyploid organisms. For site C the effect is different. If more than one A_x adaptor site exists in the genome then amino acid A_x can still be placed in locus X but it will be placed in Y as well.

Certain double mutations will have different effects. For example, if site C of the aRNA specifying amino acid A_x mutates to A_y and simultaneously A_y to A_x, the placement of all amino acids is still fixed. Locus X will now contain only A_y, and Y only A_x.

All the effects discussed and summarized in Table 1 are for an organism at a stage sufficiently late in evolution that all possible codons correspond to some amino acid. However, early in evolution an alteration may take place in site C of an adaptor molecule changing it so that it matches no existing mRNA codon. The organism will synthesize the new adaptor which will not, however, be used in protein synthesis. Moreover if the organism is haploid the amino acid corresponding to the wild-type adaptor will no longer be incorporated into any loci since that adaptor is no longer being synthesized. The result is changed, however, if for any reason there exist several copies of the DNA corresponding to a given adaptor molecule. In this case the adaptor synthesized on the unaltered DNA will allow normal protein synthesis to occur. In time, mutations in the DNA would be expected to take place causing a new complementary codon to appear. The altered adaptor which had heretofore not participated in protein synthesis will now insert the amino acid in question at the site where the new codon appears. In this manner degeneracy of the genetic code results since now there would be more than one codon corresponding to the same amino acid.

Table 1. Changes of amino acid placement in protein after mutations in activating enzyme and adaptor RNA sites.

Site	Amino acid placed in protein locus	
	X	Y
	Wild-type	
	A_x	A_y
	Mutation	
A	A_y, A_x*	A_y
R	A_x*	A_x or A_y
E	A_y, A_x*	A_y
C	0 (haploid)	A_x or A_y
	A_x (higher ploidy)	A_x or A_y

*A_x less efficiently placed or not at all.

Recent studies on the fine structure of proteins, for example, hemoglobin (*5*), indicate that in a given protein there may be an appreciable number of amino acid loci where the substitution of one amino acid for another does not noticeably alter the biochemical activity of the protein. On the other hand, it is also known that the substitution of a single amino acid at a number of loci abolishes or greatly alters the activity of the protein.

Once all codons exist, the consequence of a mutation which permanently and completely associates an amino acid (A_x) with a different codon (previously associated with A_y) is to produce structural alterations in all proteins containing these amino acids. On the basis of what is known about living systems no organism similar to present-day organisms should be able to survive such a widespread alteration of virtually all its working parts. Not only would the overall physicochemical properties of the proteins be altered, but also critical loci (not necessarily all in the active site) would probably be changed in many proteins (*6*). In order for the organism to survive there would have to be compensatory mutations, essentially in one generation, causing the critical mRNA codons to correspond to the new A_x code. The probability of this occurring is the probability of a particular nucleotide changing to another particular nucleotide, taken to a power equal to the number of different critical A_x or A_y, or both, amino acid loci in

the organism. The number of such loci does not have to be large for this probability to be so small that the event can be, for practical purposes, considered never to have occurred.

The alternative to an abrupt code change is a gradual one. However, because part of the code is expressed as independent codons throughout the genome there is no way in which an organism could gradually change from one code to another without passing through a random phase in which more than one amino acid can be placed in a locus. It is hard to imagine any circumstance under which a selective advantage would be gained by the random placement of certain protein amino acids. In fact, a change of this kind would almost certainly have large scale deleterious effects on any organism and therefore the change would not be perpetuated. Thus, once established, the genetic code will never change, barring an incredible event, and all organisms descendant from a given organism having the complete code will all have the same code.

We have postulated that the C site on the adaptor molecule cannot change permanently because it must match large numbers of codons. The A site of the aa-enzyme is also restricted because it must match, possibly with varying efficiency, amino acids which are invariant in structure. Therefore, site A (and its amino acid) and C remain permanently associated as if their relationship were chemically determined. The R site of the aa-enzyme and the E site of the adaptor molecule are, however, not restricted in this sense, since as long as the two sites change together the system will operate. Thus a mutation taking place in the R site may slightly change its fit to the existing E site. A subsequent mutation in the E site may then restore the previous mutual relationship. In this manner the genetic code remains invariant while the R and E sites change as if in unison. Differences in the R and E sites in different species would therefore be expected, and in fact are found (*3*). It is also possible that one aa-enzyme could attach a given amino acid to more than one of the aRNAs coding for that amino acid. In other words, in degenerate situations one aa-enzyme could attach a given amino acid to several different aRNAs coding for that amino acid regardless of the nature of the C site.

Because the genetic code should remain invariant, its constancy can be used to establish the number of primordial ancestors from which all (present) organisms are derived. If, for example, the code is universal, and there is evidence that it might be (*7*), then all existing organisms would be descendants of a single organism or species. If the code is not universal, the number of different codes should represent the number of different primordial ancestors that either existed during the time the present code was being completed, or existed when organisms were so simple that changes in practically all proteins were not always fatal. In either case, if different codes do exist they should be associated with major taxonomic groups such as phyla or kingdoms that have their roots far in the past.

An alternative explanation for a universal code is that the specific association between a given amino acid and its codon is determined by chemical phenomena such as those, for example, which lead to the association in a dipolar form of two atoms of hydrogen and one of oxygen to form a molecule of water. Clearly the structure of water need not be genetically prescribed. Although a chemically determined code cannot be ruled out there is little evidence for it.

RALPH T. HINEGARDNER
Department of Zoology, Columbia University, New York

JOSEPH ENGELBERG
Department of Physiology and Biophysics, University of Kentucky Medical College, Lexington

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Antibacterial and Enzymic Basic Proteins from Leukocyte Lysosomes: Separation and Identification

Abstract. *A lysosomal fraction from polymorphonuclear leukocytes has been directly analyzed for basic proteins by zone electrophoresis. Evidence for the presence of bactericidal basic proteins in polymorphonuclear leukocyte lysosomes is presented.*

Over the past decades various workers have reported bactericidal activity in extracts of polymorphonuclear leukocytes (PMN) (*1*). As a result of de Duve's description of mammalian cell lysosomes, attention has been drawn towards the study of PMN granules, which disappear during phagocytosis. Cohn and Hirsch (*2*) have reported, in rabbit PMN granules, the localization of several hydrolytic enzymes including ribonuclease, deoxyribonuclease, and lysozyme (which are basic or cationic, proteins) as well as the bulk of a bactericidal principle, "phagocytin." Using histochemical techniques, Spitznagel and Chi (*3*) have presented evidence that the antibacterial action of PMN during phagocytosis and degranulation may be due to transfer of basic proteins from PMN lysosomes to ingested bacterial cells. Purification and separation of the antibacterial substances from the enzymic basic proteins of PMN lysosomes has not yet been achieved; hence the precise physical and chemical properties, and indeed the mode of antibacterial action of substances from the PMN lysosomes, remain to be defined.

Using zone electrophoresis we have resolved the PMN lysosomes into several components with recognizable biological and biochemical properties. The components studied include three which possess both bactericidal activity and the electrophoretic mobilities of highly basic proteins.

A lysosomal fraction was prepared from guinea-pig peritoneal exudates induced by injection of 0.5 percent