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ORIGIN OF THE GENETIC CODE

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ABSTRACT

Data relating to the question of the origin of the genetic code are re-
viewed. It is considered likely that the primordial code(s) employed only
two bases, I and U. A doublet code evolved in which coding specificity
was confined to bases 1 and 2 of codons, the third base acting as a non-
specific stabiliser. The primary interactions which first established a
'genetic' code probably employed a few amino acids only - those which could
be accommodated by early transfer RNA-like polymers without the intervention
of charging enzymes. However these early 'stereochemical' interactions
need not necessarily be reflected in the structure of the contemporary code.
The ordered structure of the modern code is considered to have arisen
largely from stochastic processes.

INTRODUCTION

Even before the list of trinucleotide assignments had been
completed certain regularities in the structure of the genetic
code had generated a number of theories as to the code's origin
(Jukes 1965, Woese 1965). Today, although the structure of the
code has passed into the common body of scientific knowledge,
the question of its origin remains a puzzle. Two general
hypotheses have been advanced. The stochastic model was made
possible by the adaptor hypothesis (Crick 1958), which seemed
to separate amino acids and polynucleotides as 'complementary'
entities. This model stresses the fitness of the contemporary
code to buffer the phenotype of organisms against the effects
of harmful mutation (Sonneborn 1965) and to increase the
reliability of information transfer (Goldberg and Wittes 1966).
The stereochemical model (see Woese 1967) implies that certain
features of the code were uniquely specified by the physical
nature of the first interacting components. Both theories
have usually been presented as black and white alternatives.
The fact that they are not mutually exclusive has seldom been
explicitly stated (Reanney and Ralph 1967, Crick 1968).

THE GENETIC CODE CAN BE PRESENTED AS THE COMPLEMENT
OF THE CODE ON mRNA

The genetic code can be expressed in three ways:

1. as the triplet of bases on DNA which specifies the
complementary codon on mRNA,
2. as the codon on mRNA which interacts with the anti-
codon, of tRNA, and
3. as the anticodon of tRNA.

All of these are formally valid representations of the
molecular specificity of the code.   The reasons why the code
is usually written in terms of codons on mRNA are probably
historical and may reflect the experimental techniques used to
elucidate codons (for example the triplet binding assay used
tRNAs of (largely) unknown primary structure; the known base
sequences were those of the mRNA triplets).   However in
discussions of the code's origin it is more logical to rep-
resent the code in terms of the triplet of bases on tRNA, as
the polynucleotides which interact with amino acids are tRNAs.

In Table 1 the code is presented in terms of the anticod-
ons on tRNA written 3' → 5' to facilitate comparison with more
usual coding catalogues.   The bases in position 3 of anti-
codons are those known to occur in this position from determined
primary structures of tRNAs and those predicted by the wobble
hypothesis (Crick 1966).

TABLE 1.   THE GENETIC CODE EXPRESSED IN TERMS OF THE CODING TRIPLETS ON
           tRNA

1st position	2nd position				3rd position	
	U	C	A	G	deaminated	aminated
U	ASN	SER	ILE	THR	I	
	ASN	SER	ILE	THR		G
	LYS	ARG	ILE	THR	U	
	LYS	ARG	MET	THR		C
C	ASP	GLY	VAL	ALA	I	
	ASP	GLY	VAL	ALA		G
	GLU	GLY	VAL	ALA	U	
	GLU	GLY	VAL	ALA		C
A	TYR	CYS	PHE	SER	I	
	TYR	CYS	PHE	SER		G
	TER	TER	LEU	SER	U	
	TER	TRP	LEU	SER		C
G	HIS	ARG	LEU	PRO	I	
	HIS	ARG	LEU	PRO		G
	GLN	ARG	LEU	PRO	U	
	GLN	ARG	LEU	PRO		C

Wobble pairing in base 3 (Crick 1966)

Codon	Anticodon
U	
C	I
A	
G	C
U	
C	G
A	
G	U

Note that according to this representation, inosine (I) is a letter in the alphabet of the code.

NO PHYSICAL EVIDENCE ON THE CODE'S ORIGIN IS LIKELY TO BE FOUND IN MOLECULAR PALEONTOLOGY

Recent developments in molecular paleontology make it possible to extend the direct study of trace remains of living things far back into the pre-Cambrian era.   The oldest rocks investigated so far are the Fig Tree Chert series from South Africa (3100 million years) and the Onverwacht series, also in South Africa (3200 million years).

Engel *et al.* (1968) have found carbonaceous alga-like bodies in rocks of the Onverwacht series;   they claim that these traces represent biological remains over 3.2 billion years old.   These and other similar claims for ancient rocks have been treated with some scepticism by the scientific community because of the difficulties of unambiguously identifying any micro-fossil or chemical residue as 'biological' (see Calvin 1969).   However, many, if not most workers in this field would agree with Engel *et al.* in their conclusions that "if the carbonaceous forms (in the Onverwacht series) are fossils, the origin of unicellular life presumably has occurred in still older rocks destroyed by superimposed igneous and metamorphic episodes in the evolving earth".

SOME EVIDENCE ON THE CODE'S ORIGIN MAY BE FROZEN IN THE BIOCHEMISTRY OF CONTEMPORARY ORGANISMS

In some cases enzymatic and other functions can be altered by mutation without a lethal effect upon the cell concerned. However if a given function has a large enough number of dependent reactions then any change in the basic function cannot be tolerated.

This gives rise to a concept of 'evolutionary conservatism' (Eck and Dayhoff 1966).   Since the entire biochemistry of the cell depends upon the production, through the translation mechanism, of functional enzymes, it follows from the 'conservatism' principle that many basic features of the translation system cannot have changed throughout the measurable course of evolution.   The code itself has probably been 'frozen' in a form rather similar to its present form for even longer, for similar reasons.

Thus it is not unreasonable to expect that some features of the most primitive amino acid-polynucleotide interactions survive as 'molecular fossils' in the most intimate biochemical reactions of cells and in the structure of the code itself.

THE NATURE OF EARLY CODES

ONLY A FEW AMINO ACIDS WERE LIKELY TO HAVE BEEN CODED IN ANCIENT CODES

While it is difficult to assess the significance of abiotic synthesis data, it does seem that certain amino acids are formed more readily than others.   Of the amino acids

formed in typical experiments (Calvin 1969), the most common
products from two independent reactions were GLY, ALA and the
dicarboxylic acids, GLU and ASP.   GLY, ALA, CYS, SER, ASP and
VAL are perhaps the most thermodynamically stable of the amino
acids (Eck and Dayhoff 1966).   It is interesting that the 6
protein amino acids detected in the Murchison meteorite were GLY,
ALA, GLU, ASP, VAL and PRO (Kvenvolden, Lawless and Ponnamperuma
1971).   GLY, ALA, GLU and ASP are among the most abundant
constituents of contemporary proteins.

It is widely agreed that TRP, MET and the amides of the
dicarboxylic acids entered relatively late into the structure of
proteins (Jukes 1965, Crick 1968).

IN INTERMEDIATE CODES 2 BASES ONLY MAY HAVE BEEN REQUIRED FOR AMINO ACID RECOGNITION

In the contemporary code, degeneracy is confined to the
third letter.   Jukes (1965) suggested that the archetypal code
may have been a doublet code.   The possibility that doublets
alone can specify amino acids has been confirmed for SER by
Rottman and Nirenberg (1966) who showed that the dinucleotide
pUpC promoted the binding of SER tRNA to ribosomes in the
triplet binding assay.   There is now abundant evidence that
individual tRNA species can recognise synonym codes differing
only in the third letter and a theoretical basis for the
observed pattern of degeneracy has been proposed by Crick (1966).

Careful examination of the data used to construct the
present coding assignments reveals doublet regularities more
extensive than those normally recognised (see below).   These
support the concept that in earlier forms of the genetic code
the third base of the triplet was used solely as a stabilising
base (Reanney and Ralph 1966).   This being so, it is difficult
to avoid the inference that less sophisticated codes used only
two bases for specific recognition of their complements on
mRNA.

Söll *et al*. (1965) showed that when challenged in the
triplet binding assay with a variety of triplets, the 15-16
amino acids which are fairly widely accepted as 'primitive'
(see Jukes 1965) gave 83 'significant' stimulations.   Many
triplets stimulated the binding of more than one amino acid.
Reanney and Ralph (1966) were able to account for 81 of these
in vitro binding stimulations on the basis of a doublet code.

The doublet code in Table 2 is derived from the coding as-
signments of Brimacombe *et al*. (1965) and Söll *et al*. (1965).   In
Table 2A the first two bases shown (the specificity doublet)
are invariant while the third base (N) may vary.

Triplets in which the standard pattern of degeneracy in
position 3 is preserved account for 54 of the 83 'significant'
stimulations in Söll's assays.   Certain triplets in which
the specificity doublet occurs in position 1 and 2 do not fit
the currently accepted pattern of degeneracy in place 3, e.g.
UGA for CYS (standard codons UG-pyrimidine) and AAU for LYS
(standard codons AA-purine).   These triplets account for an
additional 4 binding stimulations (Söll *et al*. 1965).

TABLE 2. INTERMEDIATE CODES REQUIRING TWO BASES ONLY FOR AMINO ACID RECOG-
NITION

A. THE DOUBLET CODE

SPECIFICITY DOUBLET	AMINO ACID	SPECIFICITY DOUBLET	AMINO ACID
UUN	PHe and LEU	AUN	ILE
UCN	SER*	ACN	THR*
UAN	TYR or Terminate	AAN	LYS
UGN	CYS	AGN	SER and ARG
CUN	LEU*	GUN	VAL*
CCN	PRO*	GCN	ALA*
CAN	HIS	GAN	GLU and ASP
CGN	ARG*	GGN	GLY*

* amino acids with 4 place degeneracy

AMINO ACIDS NOT CODED FOR IN A DOUBLET CODE[1]

AUG	MET
UGG	TRP
CA (A,G)	GLN
AA (A,C)	ASN

1 These amino acids are thought to have evolved later than those shown in the doublet code (see Jukes 1965).

B. SPECIFICITY DOUBLET: BASES 2 AND 3, DEGENERACY IN BASE 1

ALA	AGC		GLU	UGA		VAL	CGU		GLY	(CGG)
	UGC 1			GGA			UGU			UGG
	CGC 1			AGA 1		LYS	(AAA)			CGG
ARG	CCG		HIS	UCA			UAA			AGG
	GCG			CCA						
ASP	UGA		THR	CAC						
	GGA			AAC						

1 See Nirenberg *et al*. 1965.
All other assignments from Söll *et al*. 1965.

C. INVERTED SPECIFICITY DOUBLETS

ARG	GGC	CYS	CGU
	UGA	TYR	CAU
ASP	UAG		
	CAG		

All assignments are from Söll *et al*.

A further 17 binding stimulations in Söll's assays may be accounted for on the assumption that the specificity doublet occurs in positions 2 and 3, rather than 1 and 2 (Table 2B). While the position of the specificity doublet is altered in these triplets, the specificity of any doublet for its cognate amino acids remains unchanged from that in Table 2A.

Triplets with first or third letter degeneracy account for
75 of the triplets in Söll's table.   This leaves a residue of
8 anomalous stimulations.   However, 6 of these anomalous
triplets contain specificity doublets in which the position of
the bases is inverted (Table 2C).   The stimulation of ASP
binding by UAG and CAG (normal codons 5'GAU 3' or 5' GAC 3')
might then become intelligible.   This stimulation could arise
if the conditions of the *in vitro* binding assay permit some
tRNAs to pair with triplets in a parallel fashion instead of
in the usual antiparallel manner.   The fact that inverted
doublets give relatively weak stimulation may imply that this
type of binding is less favourable than binding with anti-
parallel polarity.

The trinucleotide binding assay is open to a number of
criticisms.   In particular, depending on the Mg^{++} concentration,
one triplet can stimulate binding of several aminoacyl tRNAs;
thus the test enhances ambiguity (Khorana 1965).   It should
also be emphasized that a triplet with 5' and 3' termini with-
in the same codon represents an artificial situation the cell
is unlikely to encounter.   However, the fact that the di-
nucleotide pUpC would bind only SER tRNA (Rottman and
Nirenberg 1966) (UC being the specificity doublet for SER) and
not LEU or ILE whose codons contain UC in the second and third
positions, suggests that the triplet binding test is adequate
to cope with specific doublet recognition even though variation
in the position and polarity of the doublet is permitted under
assay conditions.

STILL EARLIER CODES MAY HAVE CONTAINED ONLY TWO BASES

The data presented in Table 2 suggest that intermediate
codes selected among amino acid R groups by employing 4^2 = 16
specificity doublets, the third base being physically present
but playing no role in specificity.   If one goes further back
into the past, it is possible to conceive (Reanney and Ralph
1967, Crick 1968 *int. al.*) of very primitive nucleic acid
molecules which contained two bases only (2^2 = 4 specificity
doublets).

It should be stressed that there is no experimental supp-
ort for the claim of a two letter code;   it is simply a
plausible assumption which makes the question of the code's
origin easier to understand (Crick 1968).

If one accepts the notion of a two letter code, an
important question is which two bases may have been involved.
Some inferences can be drawn from abiotic synthesis;   it does
seem true for example that adenine is rather more readily
formed than any other base.   The resonance energy of adenine
is greater than that for any other biologically important
heterocyclic base (Pullman and Pullman 1962).   It may also be
true that, of the pyrimidines, uracil is more easily formed
than cytosine or thymine (see Pattee 1965).

Crick (1968) has suggested that the primordial nucleic
acid contained A and I, as a stable double helix can be
formed from poly A and I and the more primitive amino acids do
tend to have purines only in their specificity doublets (I
codes like G but with one less H bond).   I can be formed
readily in abiotic synthesis (see Lowe, Rees and Markham 1963)
but Crick suggests that it was perhaps formed from adenine by

deamination.

THE 2 INITIAL BASES MAY HAVE BEEN U AND I

This leads to a point which is seldom considered in discussions of the code's origin, i.e. that the unphysiological conditions needed to generate many monomers in abiotic experiments would also lead to their modification or breakdown over long periods. It is known from the chemistry of the nucleic acids that the bases are prone to deamination in a number of circumstances. Under non-oxidising conditions at acid pH, C is converted into U (Hunter and Hlynka 1937). Alkaline treatment of the purine nucleosides at high temperatures for one hour leads to 8-9% deamination (Jones, Mian and Walker 1966).

Reanney and Ralph (1967) have postulated that the two (predominant) original bases in polynucleotide were the deaminated bases U and I. Several advantages follow from this hypothesis:

1. these two bases may have been more stable (above), hence more readily available as precursors under primitive conditions than the aminated bases;
2. in contemporary biosynthetic pathways U and I precede the other nucleotides (Fig. 1); their manufacture would thus be 'cheaper' in terms of energy and enzymes (the fact that the amination which leads to C, A and G occurs after the formation of UMP and IMP might imply that this amination step arose later in the evolution of this biosynthetic pathway).
3. in certain *in vitro* situations polymers containing I will replicate whereas those containing G will not (Karstadt and Krakow 1970), perhaps because the Tm of duplex molecules containing G/C pairs prohibits effective strand separation (Beibricher and Orgel 1973).
4. a double helix of the standard Watson-Crick type can in theory be constructed from poly I and poly U or IU copolymers by invoking enol tautomerism of I (Reanney and Ralph 1967).

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                                    OROTIDYLIC                        TMP
          ───────→  OROTIC ACID ────────→    ACID    ────→  UMP <
                                                                      CMP


                                                                      AMP
          ─────→ 5-FORMAMIDO-4-AMINO-IMIDAZOLE ────→  IMP <
                                                                      GMP

                CARBOXAMIDE RIBOTIDE
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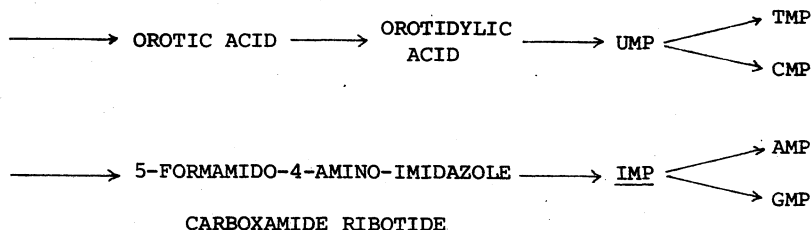
Fig. 1. Biosynthetic pathways leading to the mononucleotides.

The poly AI helix postulated by Crick suffers from the disadvantage that both A and I are purines. Orgel (1968) has pointed out that the only polynucleotides of possible evolutionary relevance are those whose complements also serve an efficient template function. In aqueous solution poly U cannot be formed on a poly A template because of the poor stacking ability of the pyrimidines (Sulston *et al.* 1968). The introduction of the pyrimidines into a predominantly A-I
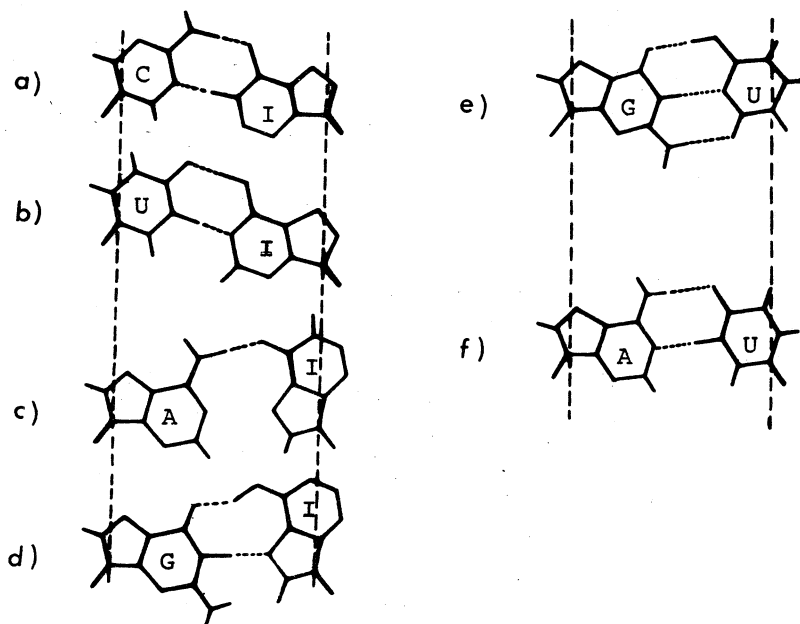
Fig. 2.   Alternative base pairs which preserve the standard interglyco-
           sidic bond distance of DNA.

Footnote to Fig. 2: DOUBLE HELICES POSSIBLE WITH U AND I WHICH PRESERVE
THE STANDARD INTERGLYCOSIDIC BOND DISTANCE OF DNA. ADAPTED FROM REANNEY
AND RALPH (1967).

Figures 2a, b, c and d show how the 4 standard nucleotides might pair with
I.   The pK of the 6 substituent of inosine (pK 8.75) is such that it is
partly ionised at pH 7.8.   The pK may be even lower than when I in a
polynucleotide is base-paired to other mononucleotides.   I can make only
a single bond with A as shown in 2c without distorting the distance between
the glycosidic bonds in the base pair.   This configuration (2c) might
permit I in a polynucleotide to bond to A in a second polynucleotide if
the bases adjacent to I were base paired.   However I in a polynucleotide
would probably not form a standard double helix with A in this configur-
ation.   I paired to A in any other way excessively distorts the
glycosidic bond distances.   The tautomeric form of I may require the
concerted approach of the appropriately oriented H bonding groups of U to
facilitate and maintain this form of bond.   Thus it is unlikely that the
tautomeric form of I would be elicited to bond to G as shown in 2d.   U is
able to form 3 H bonds with G when the enol form of U is bonded as shown
in 2e.   This does not unnecessarily distort the interglycosidic bond
distances and the resulting tautomeric form of U might be facilitated and
stabilised by the concerted action of the 2-amino and 6-keto groups of G
to which it becomes base paired.   Alternatively, as suggested for I,
stabilisation of the U-G pair might result from the existence of some
ionised U at physiological pH.

The arguments put forward here gain substance when it is remembered that the
presence of organic molecules in the environment of primitive polynucleo-
tides may have enhanced enolisation (So and Davie 1964).

helix would interrupt the geometry of the helix as the inter-
glycosidic bond distance A-I is greater than the distances
A-T(U) or I-C.   By contrast, C, A and G could be introduced
into a poly IU helix without any distortion of the molecule
(Fig. 2).   However, Arnott and Bond (1973) have shown that in
a triple stranded polynucleotide helix involving one poly A
and two poly I chains, the three helical chains have conform-
ations similar to conventional A-type double helices despite
the absence of the standard purine-pyrimidine base pairs.
They suggested that the evolution of the contemporary genetic
code from a primitive code in which A and I were the predom-
inant bases would not have required 'major discontinuities'
in molecular geometry (Arnott and Bond 1973).

   A major supporting prop for the notion that U and I were
the original bases is the inference (Reanney and Ralph 1967)
that, in cyanimide type condensations, only the deaminated
bases (especially in combination with deoxy sugars) could have
formed linear, unbranched 3' → 5' linked polynucleotides.   For
a fuller discussion of the chemistry of this point the reader
is referred to Reanney and Ralph (1967), Tener *et al.* (1958)
and Ralph and Khorana (1961).

A POLY UI CODE CAN EXPLAIN THE ORIGIN OF THE PATTERN OF DEGENERACY IN BASE 3

   Fig. 3 represents a summary of the events in the postu-
lated evolution of polynucleotides based on U and I to those
employing the 4 standard bases.   Presumably the animated bases
would have been introduced into adaptor polynucleotide over
the same time period as into template.

1.	Formation of		pI + pU
2.	Formation of template (1)	(1)	pI pU pI pU pU pI pU pI pU pU
3.	Formation of		pA + pC + pG
4.	Formation of template (2) by condensation of pU + pA + pC + pG on template (1)	(1)	pI pU pI pU pU pI pU pI pU pU
		(2)	pU pA pC pA pG pU pA pC pA pG
5.	Formation of template (3) on template (2)	(2)	pU pA pC pA pG pU pA pC pA pG
		(3)	pA pU pG pU pC pA pU pG pU pC

Fig. 3.   Summary of events during the formation, replication and
         evolution of polynucleotide templates.

   This evolution can explain in an unforced way the origin
of the present pattern of degeneracy.   To understand this,
one must look briefly at the interaction of mRNA and tRNA on
the ribosome.   As the ribosomal site(s) for tRNA must
accommodate all tRNA species, each individual tRNA must bind
in a strictly equivalent way;   specificity is confined
exclusively (?) to the H bonding interaction between codon
and anticodon.   To serve as a basis for specificity and yet
allow rapid flux of components through the ribosome the

interaction must be strong enough to hold the tRNA to the mRNA-
ribosome complex but weak enough not to hinder easy release.
The weakest interaction possible in contemporary systems is the
bonding UUU (codon) - AAG (anticodon).   This has 4 standard
H bonds and one weaker H bond.   The strongest interaction would
seem to be GGG - CCC (9 standard H bonds).   It has yet to be
proven that this latter interaction actually takes place *in vivo*.

The permitted range seems to be the bond energy of 4-5
→ 8-9 bonds.   It is a reasonable assumption that the function
of the third 'spacer' base is/was less to provide specificity
(although it does this to some extent in the contemporary code)
than to provide the extra energy necessary to stabilise the
codon-anticodon interaction in the correct Tm range.

Fig. 2 shows that U could pair ambiguously with A and G
while I could pair ambiguously with C or U(T).   As the
aminated bases became available, they would rapidly replace U
and I in the specificity doublets of codons, owing to the
possibilities for greater specificity and the introduction into
the code of new amino acids;   however such pressures would not
apply to the third base.   Retention of ambiguous pairing here
would lead to a situation where the two purines and two pyri-
midines were not discriminated in base 3 of all sense codons.

As new amino acids spread over the code some amino acids
whose role in protein structure was more crucial than most
(e.g., PRO) or whose frequency in 'statistical' protein was
high (e.g., GLY) would acquire genetic 'protection' against
mutational alteration in the form of extra codons.   This
follows from the predictions of all stochastic models of the
code's evolution (Goldberg and Wittes 1966, Crick 1968).
Four place degeneracy, i.e. four codons rather than two,
could be achieved using U and I only;   this is still a
possibility in the contemporary code (Table 1).   The retention
of I into the modern code or its re-introduction by eukaryotes
following the development of 'anticodon deaminase' follows
from the wobble pairing possible with I (Crick 1966).   C was
probably introduced into base 3 of anticodons only when the
'late' amino acids MET and TRP entered the code.   MET in
particular has come to play a unique role in the initiation
of protein biosynthesis;   its assignment of a unique codon
(AUG) would remove this triplet from the set presumably
previously occupied by ILE.   The unusual pattern of degener-
acy shown by ILE would follow from this hypothesis.   It is
interesting that the two late additions to the code are both
concerned with punctuation;   MET with initiation, TRP taking
over a codon which was probably previously a TER codon (UGG
obeys the rule: U.pu.pu in mRNA = TER).   This suggests that
there occurred at some stage of evolution a significant
modification of the system of translation.   The observation
that certain viral proteins are derived by enzymatic hydrolysis
from a much larger polypeptide suggests that primitive
chromosomes were translated as wholes and that the active
'fragments' arose from hydrolysis at 'weak links' in the
chain.   In 28 out of 29 cytochromes, the C terminal amino acids
are related to chain terminating codons by a one base change
(Nolan and Margoliash 1968).

It may be significant that the 8 amino acids with 4 place degeneracy all contain G and/or C in their specificity doublets, suggesting that extra stabilisation was required to compensate for the proposed nonstandard pairings in position 3 of triplets.

The replacement of U by T would follow from the fact that the higher pK of T, (pK 9.8) cf. U (pK 9.2), reduced the possibility of ambiguous pairing (Reanney and Ralph 1967).

PROTO tRNA

The pre-requisites for any primitive adaptor are:
1. a terminus (not necessarily CCA) to which the amino acid can be transferred, and
2. a region of complementarity (not necessarily involving the current bases) which can base pair with message.

It also seems likely that such a molecule would probably require some degree of 3D structure. Thus one is forced to the (at first sight) unlikely conclusion that even the most primitive adaptors were tolerably complicated molecules. On the basis of sequence homologies Jukes (1966) has postulated that all known tRNAs derive from a common ancestor 84 nucleotides long. It is hard to conceive of the most primitive translation systems using so elaborate a molecule. Even if sequence periodicity within present tRNAs indicates, however, that the 'ancestral' tRNA was derived by gene duplication from a smaller gene (or gene set), the requirement for tertiary structure sets a limit to the 'smallness' of the adaptor.

THE DIFFICULTY OF DISPROVING THE
STEREOCHEMICAL HYPOTHESIS

One can now invert current arguments concerning the likelihood of discovering amino acid-polynucleotide interactions. It would in fact have been surprising if any such interactions had been discovered, given the reaction conditions and components hitherto employed.

It is important to note that the stereochemical model does not require polynucleotides to recognise all 20 amino acids. In contrast with some protagonists of the stereochemical theory I believe that stereochemical interactions were important only for the few amino acids necessary to establish the system. This presupposes that most attempts to demonstrate some kind of interaction are doomed to failure at the outset. Furthermore it is by no means certain that the polymers implicated in the initial interactions resembled modern nucleic acids in terms of component bases or even in terms of the 3'—5' phosphodiester linkage (Sulston *et al*. 1968). The point of these considerations is to stress that the later evolution of the code may have obliterated many of the features on which its inception depended (but see 4).

Fresco *et al*. (1966) have convincingly demonstrated that the biological activity of tRNA can be critically affected by factors such as pH, temperature, electrolyte concentration and Mg levels. The chemical milieu in which the initial coding interactions took place was undoubtedly very different to the reaction mixtures used by modern experimentalists. The presence of a variety of organic molecules (formaldehyde,

ethanol, etc.) may have conferred upon the solution solvent
properties lacking in 'physiological' media.   For example
organic compounds such as ethanol might enhance the likeli-
hood of tautomerism in the bases (So and Davie 1964).

Thus unless it is possible to specify more exactly the
nature of the environment of the interacting components, then
it may be unrealistic to expect any clear-cut binding
specificity between amino acids (and/or their derivatives) and
polynucleotides to reveal itself.

STOCHASTIC MODELS OF THE CODE'S EVOLUTION

A number of workers have stressed that the contemporary code
is highly nonrandom.   Mackay (1967) showed that the natural
code closely approximated to a statistically constructed
'optimal' code.   Alff-Steinberger (1969) compared the amino
acid substitutions resulting from single base substitutions in
the natural code with those of substitutions in computer
generated random codes.   For a number of amino acid parameters
(molecular weight, polar requirement, pKi, etc.) it was shown
that single base substitutions in the first position of codons
tended to result in the substitution of an amino acid more
similar to the original amino acid than would be expected from
a random code.   The 'protective' features of the code have been
convincingly summarised by Goldberg and Wittes (1966).

Thus the code can be considered (in the jargon of cyber-
netics) as an error-minimising code (Alff-Steinberger 1969).
It follows that the code may have been brought into being solely
by the constant selective pressures to ensure fidelity of
translation and to buffer the phenotype of organisms against the
effects of mutation.

Features of stochastic models have been reviewed by Woese
(1967).   I will confine myself to one aspect of the code which
(to the best of my knowledge) has not been treated so far in
stochastic arguments on code evolution.

In the modern code degeneracy is confined to base 3 but an
equally 'protective' code is theoretically possible with the
redundancy shifted to base 1.   The localisation of degeneracy
in base 3 may arise from the vectorial nature of translation:
if the probability of mutation is randomly distributed along
the length of the genome, then it is advantageous to have the
two specific bases in which the identity of the amino acid
resides as close as possible to the N terminus from which peptide
synthesis starts.   This arrangement maximises the chance that
a given base sequence will be 'safely' translated before
mutation can change the identity of the amino acid(s).

It is very likely that much of the structure of the
contemporary code results from selective pressure as predicted
by stochastic models.   But while stochastic arguments can
convincingly explain the later evolution of the code, they say
nothing about its origin.   It is not easy to see how any
system of translation could have begun from the completely random
assortment of amino acid - nucleotide interactions which the
stochastic argument, in its extreme form, implies.   It is to
be hoped that some stereochemical specificities can be
experimentally demonstrated otherwise our chances of under-
standing the code's origin are remote.

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