The hypercube structure of the genetic code explains conservative and non-conservative aminoacid substitutions in vivo and in vitro

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Abstract

A representation of the genetic code as a six-dimensional Boolean hypercube is described. This structure is the result of the hierarchical order of the interaction energies of the bases in codon-anticodon recognition. In this paper it is applied to study molecular evolution in vivo and in vitro. In the first case we compared aligned positions in homologous protein sequences and found two different behaviors: (a) There are sites in which the different amino acids may be explained by one or two 'attractor nodes' (coding for the dominating amino acid(s)) and their one-bit neighbors in the codon hypercube; and (b) There are sites in which the amino acids correspond to codons located in closed paths in the hypercube. In the second case we studied the 'Sexual PCR'1 experiment described by Stemmer [Stemmer (1994)] and found that the success of this combination of usual PCR and recombination is in part due to the Gray code structure of the genetic code.

Keywords: Genetic code; Six dimensional Boolean hypercube; Molecular evolution; In vitro and in vivo

1. Introduction

The genetic code is the biochemical system for gene expression. It deals with the translation, or decoding, of information contained in the primary structure of DNA and RNA molecules into protein sequences. Therefore, the genetic code is both, a physico-chemical and a communication system.

Physically, molecular recognition depends on the degree of complementarity between the interacting molecular surfaces (by means of weak interactions); informationally, a prerequisite to define a code is the concept of distinguishability. It is the physical indistinguishability of some codon-anticodon interaction energies that makes the codons synonymous and the code degenerate and redundant (Crick, 1966).

In natural languages (Harris, 1988) as well as in the genetic code the total redundancy is due to a
hierarchy of constraints acting one upon another. The specific way in which the code departs from randomness is, by definition, its structure. It is assumed that this structure is the result of the hierarchical order of the interaction energies of the bases in codon-anticodon recognition. The hypercube structure of the genetic code as currently introduced (Jiménez-Montaño et al. 1994) will be described and its implications for molecular evolution and test-tube evolution experiments will be discussed. As we shall see the genetic code may be represented by a six-dimensional Boolean hypercube in which the codons (actually the codewords; see below) occupy the vertices (nodes) in such a way that all kinship\(^2\) neighborhoods are correctly represented. This approach is a particular application to binary sequences of length six of the general concept of sequence-space, first introduced in coding theory by Hamming (Hamming, 1950; Ebeling, 1982; Ebeling, 1977).

A code-word is next to six nodes representing codons differing in a single property. Thus the hypercube simultaneously represents the whole set of codons and keeps track of which codons are one-bit neighbors of each other. Different hyperplanes correspond to the four stages of the evolution of the code according to the Co-evolution Theory (Dillon, 1978; Wong, 1975; Wong, 1976). Transitions within three of the 'columns' (four-dimensional cubes), consisting of the codon classes NGN, NAN, NCN, and NUN, lead to silent and conservative amino acid substitutions; while transitions in the same hyperplane (four-dimensional subspace belonging to any of the codon classes ANN, CNN, GNN or UNN) lead to non-conservative substitutions as frequently found in proteins. The proposed structure demonstrates that in the genetic code there is a good balance between conservatism and innovation. To illustrate these results several examples of the non-conservative variable positions of homologous proteins are discussed. Two different behaviors were found: 1. There are sites in which the different amino acids may be explained by one or two 'attractor nodes' (coding for the dominating amino acid(s)) and their one-bit neighbors in the codon hypercube; and 2. There are sites in which the amino acids correspond to codons located in closed paths in the hypercube.

Very recently the rapid evolution of a protein in vitro by DNA shuffling has been accomplished by Stemmer (1994).

This experiment called by Smith 'Sexual PCR', was further discussed in (Smith, 1994). Smith recalls that Stemmer investigated the β-lactamase gene TEM-1 which has a very low activity against the antibiotic cephalaxine\(^3\). After three cycles of mutagenesis, recombination and selection he found the minimum inhibitory concentration to be 16 000 times higher than that of the original clone.

It will be shown that, without exception, the amino-acid replacements in TEM-1 mutants selected for high resistance to cephalaxine may be accounted by one bit changes of the corresponding codons. This shows that the structure of the code permits a very significant change in function of the coded protein by means of one-bit changes of some of the codons, provided that these mutations are integrated in a single polynucleotide by recombination.

2. Codon-anticodon interaction

The four bases occurring in DNA (RNA) macromolecules define the corresponding alphabet \(X\): \{A,C,G,T\} or \(X\): \{A,C,G,U\}. Each base is completely specified by two independent dichotomic categorizations (Fig. 1):

1. According to its chemical type \(C\): \((R,y)\) where \(R\): (A,G) are purines and \(y\): (C,U) are pyrimidines; and 2. According to H-bonding, \(H\): \{(W,S)\}, where \(W\): (A,U) are weak and \(S\): (C,G) are strong bases. The third possible partition into imino/keto bases is not independent from the former ones. Denoting by \(C_i\) the chemical type and by \(H_i\) the H-bond category of the base \(B_i\) at position \(i\) of a codon our basic assumption says that the codon-anticodon interaction energy obeys the following hierarchical order:

\[
C_2 > H_2 > C_1 > H_1 > C_3 > H_3
\]

\(^2\) The term kinship means the relationship between members of the same family.

\(^3\) The minimum inhibitory concentration for Escherichia coli bacteria carrying TEM-1-bearing plasmid is only 20 ng ml\(^{-1}\).
These life-times are influenced by the nature of the pairs: they are shorter for A–T than for G–C pairs (Guéron, 1990).

The bases are represented by the nodes of a 2-cube (Fig. 1). The first attribute is the chemical character and the second one is the hydrogen-bond character. Extending this association to base triplets, each codon is associated in a unique way with a codeword consisting of six attribute values (see Table 1).

In some of the hypercube directions single feature codon changes (one-bit code-word changes) produce synonymous or conservative amino acid substitutions in the corresponding protein (when the transitions occur in three of the four cubes displayed as 'columns' in Figs. 2 and 4); while in other directions lead to context dependent replacements which, in general, conserve only certain physical properties. However, if these properties are the only relevant ones in the given context, the substitution has little effect on the protein structure as well. These low-constraint sites facilitate evolution because they allow the transit between hypercube columns belonging to amino acids with very different physico-chemical properties (e.g. hydrophobic and hydrophilic amino acids, respectively).

3. Gray code structure of the genetic code

An n-dimensional hypercube, denoted by $Q_n$, consists of $2^n$ nodes each addressed by a unique n-bit identification number. A link exists between two nodes of $Q_n$ if and only if their node addresses differ in exactly one bit position. A link is said to be along dimension $i$ if it connects two nodes which addresses differ to as the $i$-th bit (where the least significant bit is referred to as the 0th bit). $Q_3$ is illustrated in Fig. 4. Two nodes in a hypercube are said to be adjacent if there is a link between them. The (Hamming) distance between any two cube nodes is the number of bits differing in their addresses. The number of transitions needed to reach a node from another node equals the distance between the two nodes. A d-dimensional sub-cube in $Q_n$ involves $2^d$ nodes which addresses belong to a sequence of $n$ symbols $\{0,1,*\}$ in which exactly $d$ of them are of the symbol * (i.e. the don't care symbol which value can be 0 or 1).
Table 1
Gray code representation of the genetic code

| 0 0 0 0 | 1 1 A A C N |
| 0 0 0 0 | 0 0 A A U N |
| 0 0 0 0 | 0 0 A A A K |
| 0 0 0 0 | 0 1 A A G K |
| 1 0 0 0 | 0 0 A A G Y |
| 1 0 0 0 | 0 0 A A A Y |
| 1 0 0 0 | 0 0 A A H C |
| 1 0 0 0 | 0 0 A A H H |
| 1 0 0 0 | 0 0 C A Q Q |
| 1 0 0 0 | 0 0 C A G A |
| 0 0 0 0 | 0 0 G A G E |
| 0 0 0 0 | 0 0 G A A E |
| 0 0 0 0 | 0 0 G U U U |
| 0 0 0 0 | 0 0 G U A V |
| 0 0 0 0 | 0 0 G U G L |
| 0 0 0 0 | 0 1 C U A L |
| 0 0 0 0 | 0 1 C U L L |
| 0 0 0 0 | 0 0 U U F F |
| 0 0 0 0 | 0 0 U U A L |
| 0 0 0 0 | 0 0 U U C M |
| 0 0 0 0 | 0 0 A U A I |
| 0 0 0 0 | 0 0 A U C I |
| 0 0 0 0 | 0 0 A C C I |
| 0 0 0 0 | 0 0 A C U T |
| 0 0 0 0 | 0 0 A C C T |
| 0 0 0 0 | 0 0 A U A T |
| 0 0 0 0 | 0 0 A U U T |
| 0 0 0 0 | 0 0 U U C T |
| 0 0 0 0 | 1 0 C C P P |
| 0 0 0 0 | 1 0 C C G G |
| 0 0 0 0 | 0 0 G C A A |
| 0 0 0 0 | 0 0 G C U A |
| 0 0 0 0 | 0 0 G C C A |
| 0 0 0 0 | 0 0 G C C G |
| 0 0 0 0 | 0 0 G G U G |
| 0 0 0 0 | 0 0 G G A G |
| 0 0 0 0 | 0 0 G G G G |
| 1 0 0 0 | 0 0 C G G R |
| 1 0 0 0 | 0 0 C G A R |

The first six columns represent the six-dimensional vectors (code-words). Then follow the corresponding codons. Finally the amino acids in single letter notation. The first two digits correspond to the first base, the following two to the second base and the last two to the last base according to the binary codification of the bases of Fig. 1.

Fig. 2. The six-dimensional hypercube. Each node is labeled with the corresponding amino acid in the single letter notation or terminator symbol. The thick, dashed lines represent a complex connection between two (three-dimensional) cubes. Such a line represents eight edges each, connecting the corresponding nodes of two neighboring three-dimensional cubes (see Fig. 3). The cluster of amino acids of the first example discussed in the text is displayed by fat points at the corresponding nodes and dashed thin curved lines for the edges.
The idea to propose a Gray Code representation of the Genetic Code goes back to Swanson (1984) where this concept is explained in detail (see also, Jiménez-Montaño, 1994). However, a great number of different Gray Codes can be associated to the Genetic Code depending on the order of importance of the bits in a code-word. In Table 1 our chosen Gray Code is displayed. It is constructed according to our main hypothesis:

\[ C_2 > H_2 > C_1 > H_1 > C_3 > H_3 \]

For example, the first two lines of the table differ in the last bit corresponding to \( H_3 \); which is the least significant bit; the second and the third lines differ in the next least significant bit, i.e. \( C_3 \), and so forth.

4. The structure of codon doublets

This section is more mathematical than the rest of the paper. It is not essential for the understanding of the rest of the paper.

In a pioneering paper Danckwerts and Neubert (1975) discussed the symmetries of the sixteen \( B_1B_2 \) codon doublets in terms of the Klein-4 group of base transformations. Here their result will be recast in a form of a decision-tree (Fig. 5) and their analysis will be extended to the \( B_3B_3 \) doublets. They found the following structure for the set \( M \) of \( B_1B_2 \) doublets:

Starting from \( A_e \) generate the set:

\[ M_0 = (((1,1) \cup (\alpha,1) \cup (\alpha,\beta) \cup (\alpha,\gamma))AC) \]
\[ = \{AC,CC,CG,CU\} \]

\[ M_1 = [(1,1) \cup (\beta,1)] M_0 \]

\[ M_2 = (\alpha,\alpha) M_1 \]

![Decision-tree](image_url)

Fig. 5. Decision-tree of codon categories and redundancy distribution. The leaves are the sets of four-fold \( M_1 \) and less than four-fold \( M_2 \) degenerate \( B_1B_2 \) doublets.

![Hypercube representation](image_url)

Fig. 4. The hypercube representation of the genetic code. Each node represents a code-word (six-dimensional vector) of attribute values. However, for clarity of interpretation, the nodes are labeled with the corresponding codons (See Table 1 for the assignment of codons to vectors). The nodes and links mentioned in second example discussed in the text are shown. The edges connect: AGG ← AGC, AGC ← ACC, AGC ← AAC, UCC ← ACC ← ACC ← GCC, GCC ← CCC, CGC ← CAC, CAC ← CAG, CAC ← CUC, CAC ← GAC

![3D visualization](image_url)

Fig. 3. Each of the fat short dashed lines represent eight edges, connecting the corresponding nodes of two three-dimensional cubes. The figure shows a four-dimensional cube using the symbolic fat drawn link (top) and the same cube using standard representation.
The sets $M_1$ and $M_2$ consist of four-fold and less than four-fold degenerate doublets, respectively. The set $M$ can be expressed as:

$$M = ((1,1) \cup (\beta,1)) \cup ((1,1) \cup (\alpha,\alpha)) \ M_0$$

Where the base exchange operators $\alpha, \beta, \gamma$ are defined in Fig. 1. They showed that: $M_1$ and $M_2$ are invariant by operating with $\beta(1)$ on $B_1$, but no operation on $B_2$ leaves $M_1$ or $M_2$ invariant. Thus $B_2$ carries more information than $B_1$ and $B_2$ is therefore more important for the stability of $M_1$ and $M_2$ than $B_1$. A change of $B_1$ with respect to its hydrogen bond property does not change the resulting amino acids if all doublets of either $M_1$ or $M_2$ are affected.

Reversing supposition and conclusion, $M_1$ and $M_2$ may be defined as those doublet sets of eight elements which are invariant under the $(\beta,1)$-transformation. Then experience shows that $M_1$ and $M_2$ are fourfold and less than fourfold degenerate, respectively.

Thus the third base degeneracy of a codon does not depend on the exact base $B_1$, but only on its H-bond property (weak or strong).

The above results can simply be visualized as a decision-tree (Fig. 5). It can be seen from this figure that redundancy of a codon is determined only by the H-bond character of $B_1$ and $B_2$: SSN codons (with six H-bonds in $B_1B_2$) belong to $M_1$ while WWN codons (with four H-bonds in $B_1B_2$) belong to $M_2$. However, for codons WSN and SWN (with five H-bonds in $B_1B_2$) it is not possible to decide unless one has more information about the second base: WCN and SUN belong to $M_1$ while WGN and SAN belong to $M_2$. In all cases at most three attributes are necessary to determine the redundancy of a codon up to this point. Of course the non-degenerate codons (UAG for Methionine and UGG for Tryptophan) will require the specification of the six attributes.

From the decision rules obtained from Fig. 5 it is clear that there are branches where the refinement procedure cannot continue (the branches which end in $M_1$) because no matter which base occupies the third codon position the degeneracy cannot be lifted. This imposes a limit to the maximum number of amino acids which can be incorporated to the code without recurring to a 'frozen accident' hypothesis. Our proposal generalizes the 'two-out-of-three' hypothesis of Lagerkvist (1978) which refers only to codons in the SSN class.

The 16 $B_1B_2$ doublets can be represented as the vertices of a four-dimensional hypercube. Fig. 6 shows that the sets $M_1$ and $M_2$ are located in compact regions. Notice that this figure differs from the one introduced by Bertman and Jungck (1979) who considered as basic transformations $\alpha$ and $\beta$, instead of $\beta$ and $\gamma$ as we did. Since the operator $\alpha$ changes two bits we do not consider it as basic.

Let's consider now the structure of the set $M'$ of $B_2B_3$ doublets: Exactly as before define the sets

$M'_0 = \{ NC \}$
$M'_1 = ((1,1) \cup (1,\beta)) \ M'_0$
$M'_2 = (\alpha,\alpha) \ M'_1$ (alternatively $M'_1 = (\alpha,\alpha) \ M'_2$),

where $M'_1$ consists of the doublets $B_2B_3$ ending in a strong base (NS) and $M'_2$ of the doublets ending in a weak base (NW).

Then

$$M' = M'_1 \cup M'_2$$

can be expressed as

$$M' = ((1,1) \cup (1,\beta)) \cup (1,1) \cup (\alpha,\alpha) \ M'_0$$

Notice that the operator acting on $M'_0$ has the same functional form as the operator acting on $M_0$ above, except that $\beta$ acts as the third base instead of the first.

The sets $M'_1$ and $M'_2$ are invariant under the $(1,\beta)$-transformations. Then experience shows that the 32 codons in the class $NB_2B_3$, with $B_2B_3$ in $M'_1$ or $M'_2$ constitute a complete code codifying for the 20 amino acids and terminator signal (stop-codon), if allowance is made for deviating codon-assignments found in Mitochondria (Jukes, 1983). For the codons in $M'_1$ this is true in the universal code; for codons in $M'_2$ AUA should codify for $M$ instead of T and UGA for $W$ instead of stop signal. Both changes have been observed in Mitochondria. This more symmetric code has been considered more similar to an archetypal code.
than the universal code (Jukes, 1983). Only after
the last attribute \( H_3 \) was introduced the universal
code was obtained, with the split of AUR into
AUA (\( J \)) and AUG (\( M \)) and UGR into UGG (\( W \))
and UGA (\( i \)).

It has been speculated that primordial genes
could be included in a 0.55 kb open reading frame
(Naora, 1987). The same authors calculated that
with two stop codons this open reading frmes
would have appeared too frequently. From the

present view the assignment of UGA to a stop
codon was a late event that optimized this frequen-
cy (this interpretation differs from the one proposed
in (Naora, 1987; Brentani, 1990) where a
primordial code with three stop codons is assumed.
Other deviations of the universal code most
likely also occurred in the last stages of the code’s
evolution.

In the same way as before the sixteen \( B_2 B_3 \)
doublets can be represented as the vertices of a
four-dimensional hypercube (Fig. 7). The sets
\( M'_{1}\) and \( M'_{2}\) are also located in compact regions.
Codons with \( B_2 B_3 \) in \( M'_{1}\) are frequently used in
eukaryotes. In contrary, codons with \( B_2 B_3 \) in \( M'_{2}\)
are frequently used in prokaryots. The described
structure of the code allows a modulation of the
codon-anticodon interaction energy (Grosjean et
al., 1978; Jimenez-Montaño, 1994).

5. Examples

Besides the results mentioned in the last section
which refer to codon doublets, to further illustrate
the significance of proposed approach, we are
going to consider several examples of molecular
evolution.

The first example (Fig. 2) refers to the alignment
studied using the method of hierarchical analysis
of residue conservation by Livingstone and Barton
(Fig. 2 in Livingstone, 1993). In position 11 appear
the following amino acids \( R, W, H, G, D \), which
according to their approach have no properties in
common. In Fig. 2 this cluster of amino acids is
shown. By looking at the Atlas of amino acid
properties (Nakai, 1988) we see that from the proper-
ties proposed by Grantham (Grantham, 1974)
(composition, polarity and volume) apparently the
only requirement for the amino acids at this site is
to maintain a certain degree of polarity. From this
observation we may conclude that most probably
it is an external site. Simply by looking at such a
diverse set of amino acids one can hardly realize
that they have clustered codons. This clustering
facilitates the occurrence of mutations that in the
course of evolution were fixed, in view of the low
physico-chemical requirements at the site.
As a second example (Fig. 4) let us consider site 33 of the alignment of 67 SH2 domains, Fig. 6 of (Livingstone and Barton, 1993). We can see from Fig. 4 that the cluster around the codon CAC (H) explains, by one-bit changes, the amino acids R, Q, L, H, D. Furthermore, a second cluster around the codon AGC (S) explains the amino acids R, N, S, T. Finally, a silent change from AGC (S) to UCC (5) accounts for the minor appearance of the small, neutral amino acids, A, T, P/. In a similar way the variation of the hyper-variable region of immunoglobulin kappa light FR1 at position 18 can be explained (Fig. 8). The number after the amino acid symbol in Fig. 8 is the number of times the amino acid occurs in the alignment in Kabat (1991).

As a third example consider the residue frequencies in 226 globins displayed in Table 3 of the paper by Bashford et al. (Bashford et al. 1987). From this table we find that there are variable positions in which one or two residues predominately occur and the rest are only marginally represented and others in which the frequencies are more evenly distributed among the amino acids. As it can be easily shown, the first class of positions may be associated, at the codon level, with one (or two) attractor node(s) and its one-bit neighbors. The second one can be associated with closed trajectories in the hypercube. The corresponding figures are not included because of lack of space.

Finally, let us discuss the 'sexual PCR' experiment. In the paper by Smith (Smith, 1994) a table is displayed showing the positions in the TEM-1 gene where mutations occur, together with the substitutions found in the variant genes ST-1, ST-2 and ST-4 which show increased resistance to cefotaxime. We refer to the mentioned paper for further details. Locating these mutations in the hypercube (Fig. 4) one can easily convince oneself that all mutations may be accounted by one-bit changes at the codon level. Therefore, only six codons (four or five amino acids) are searched in each mutation and not 19 alternatives. This finding helps to explain why this in vitro realization of a 'genetic algorithm' was so successful.

It is well known in the field of Genetic Algorithms that a proper encoding is crucial to the success of an algorithm. Furthermore in (Caruna, 1988) it is shown the superiority of Gray coding over binary coding for the performance of a genetic algorithm. As it was shown above the structure of the genetic code is precisely the structure of a Gray code. Therefore, it is our claim that this is one of the reasons why very efficient variants were found after very few rounds of recombination. Most probably other reasons are: the initial population was not random, but consisted of selected sequences and these sequences were very similar among themselves. This explanation of the results of Stemmer's experiment differs from the explanation advanced by Smith (Smith, 1994).

6. Concluding remarks

The present approach goes beyond the usual analyses in terms of single base changes, because it takes into account the two characters of each base and therefore it represents one-bit changes. Besides the base position within the codon is also considered. The fact that single bit mutations occur frequently is expected from probabilistic arguments. However, one could not expect a priori, that a cluster of mutations would correspond, at the amino acid level, to a cluster of
amino acids fixed by natural selection. We have found that this situation presents itself for many positions of homologous protein sequences of many different families (results not included). The structure of the code facilitates evolution: the variation found at the variable positions of proteins does not correspond to random jumps at the codon level, but to well-defined regions of the hypercube. Finally, the Gray code structure of the genetic code helps to explain the success of 'Sexual PCR' experiments.

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