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On the Hypercube Structure of the Genetic Code

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Abstract: A representation of the genetic code as a six–dimensional Boolean hypercube is proposed. It is assumed here that this structure is the result of the hierarchical order of the interaction energies of the bases in codon–anticodon recognition. The proposed structure demonstrates that in the genetic code there is a balance between conservatism and innovation. Comparing aligned positions in homologous protein sequences two different behaviors are found: a) There are sites in which the different amino acids present 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 present correspond to codons located in closed paths in the hypercube. The structure of the code facilitates evolution: the variation found at the variable positions of proteins do not corresponds to random jumps at the codon level, but to well defined regions of the hypercube.

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 [1].

In natural languages [2] 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 here that this structure is the result of the hierarchical order of the interaction energies of the bases in codon-anticodon recognition. As we shall see, it may be represented by a six-dimensional boolean hypercube in which the codons (actually the code-words; see below) occupy the vertices (nodes) in such a way that all kinship 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 [3].

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 [4-6]. Hops 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 hops in the same hyperplane (four-dimensional subspace belonging to any of the codon classes ANN, CNN, GNN or UNN) lead to non-conservative substitutions, frequently found in proteins. The proposed structure demonstrates that in the genetic code there is a good balance between conservatism and innovation. To illustrate the results several examples of the non-conservative variable positions of homologous proteins are discussed. Two different behaviors are found: a) There are sites in which the different amino acids present 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)

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There are sites in which the amino acids present correspond to codons located in closed paths in the hypercube.

2. Codon–Anticodon Interaction

In his early paper Eigen [7] recognized that the optimization between stability and rate, that is always found for enzyme–substrate interactions, also applies to the codon–anticodon interaction. However, he attributes the codons size to mechanistic coincidences: “codons with less than three bases would be very unstable (at least for A and U). Codons with more than three bases, especially for G and C, become too ‘sticky’ ”. This is certainly not a coincidence, but a requirement for the system to function as an efficient communication device. Three bases are needed for effectively binding the adapter to the messenger. Thus, the codons size determines the range of codon–anticodon overall interaction strength within which recognition can occur¹. Genetic translation rate is limited, among other things, by codon–anticodon recognition which in turn depends on base–pair lifetimes in a given structural situation. These life–times are influenced by the nature of the pairs: they are shorter for AT than for GC pairs [9].

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

(i) according to chemical type $C : \{R, Y\}$, where R: (A, G) are purines and Y:(C, U) are pyrimidines, and (ii) according to H–bonding, $H : \{W, S\}$, where W:(A, U) are weak and S:(C, G) 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:

$$C_2 > H_2 > C_1 > H_1 > C_3 > H_3$$

This means, that the most important characteristic determining the codon–anticodon interaction is the chemical type of the base in the second position.

¹ Interestingly enough, this feature of genetic communication system has, its counterpart in human communication. In a series of experiments on reading lists of words, performed by J.E. Karlin and J.R. Pierce (Pierce J.R., An Introduction to Information Theory, Dover Publications,Inv. N.Y. 1961), in which the subject “transmits” the information translating it into the new form, speech rather than print, by reading the list aloud, they concluded that: “It seems fairly clear that reading speed is limited by word recognition not by word utterance” (underlined in the original). See also [8]

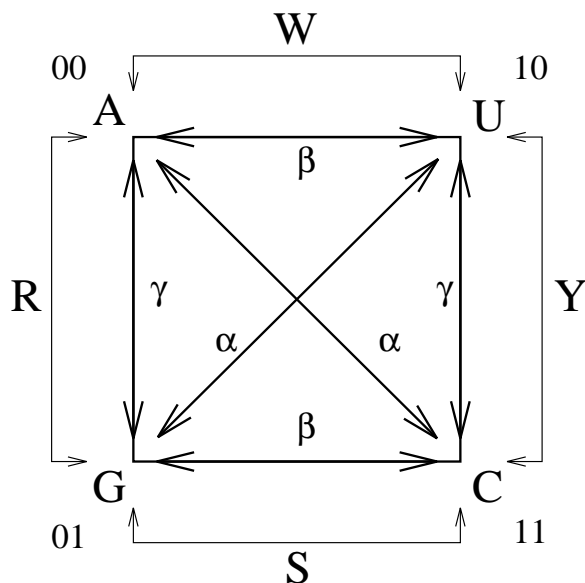


Fig. 1. Categorizations of the bases. The categorizations of the bases according to (i): chemical type $C : \{R, Y\}$, where $R: (A, G)$ are purines and $Y: (C, U)$ are pyrimidines, and (ii) according to H-bonding, $H : \{W, S\}$, where $W: (A, U)$ are weak and $S: (C, G)$ strong bases. The third possible partition into imino/keto bases is not independent on the former ones and is irrelevant for the codon-anticodon interaction. The binary representation of the bases is also shown. The first bit is the chemical type and the second one the H-bonding character. α , β and γ are the transformations of the bases, which form a Klein-4 group [6,8].

The next most important characteristic is whether there is a weak or strong base in this position, then the chemical type of the first base and so on.

The bases are represented by the nodes of a 2-cube (Fig. 1). The first attribute is the chemical character and the second the hydrogen-bond character. Extending this association to base triplets, each codon is in a unique way associated 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 hops occur in three of the 4-cubes displayed as “columns” in Figs. 2 and 3) 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).

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

Table 1

Gray code representation of the genetic code. In the first and fourth blocks the 6–binary vectors (code–words) are shown. In the second and fifth blocks appear the corresponding codons. Finally in the third and sixth columns 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

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 whose addresses differ to as the i th bit (where the least significant bit is referred to as the 0th bit). Q_6 is illustrated in Fig. 3. Two nodes in a hypercube are said to be adjacent if there is a link present between them. The (Hamming) distance between any two cube nodes is the number of bits differing in their addresses. The number of hops needed to reach a node from another node equals the distance between the two nodes. A d –dimensional subcube in Q_n involves 2^d nodes whose addresses belong to a sequence of n symbols $\{0, 1, *\}$ in which exactly d of them are of the symbol “*” (i.e. they dont care symbol whose

value can be 0 or 1).

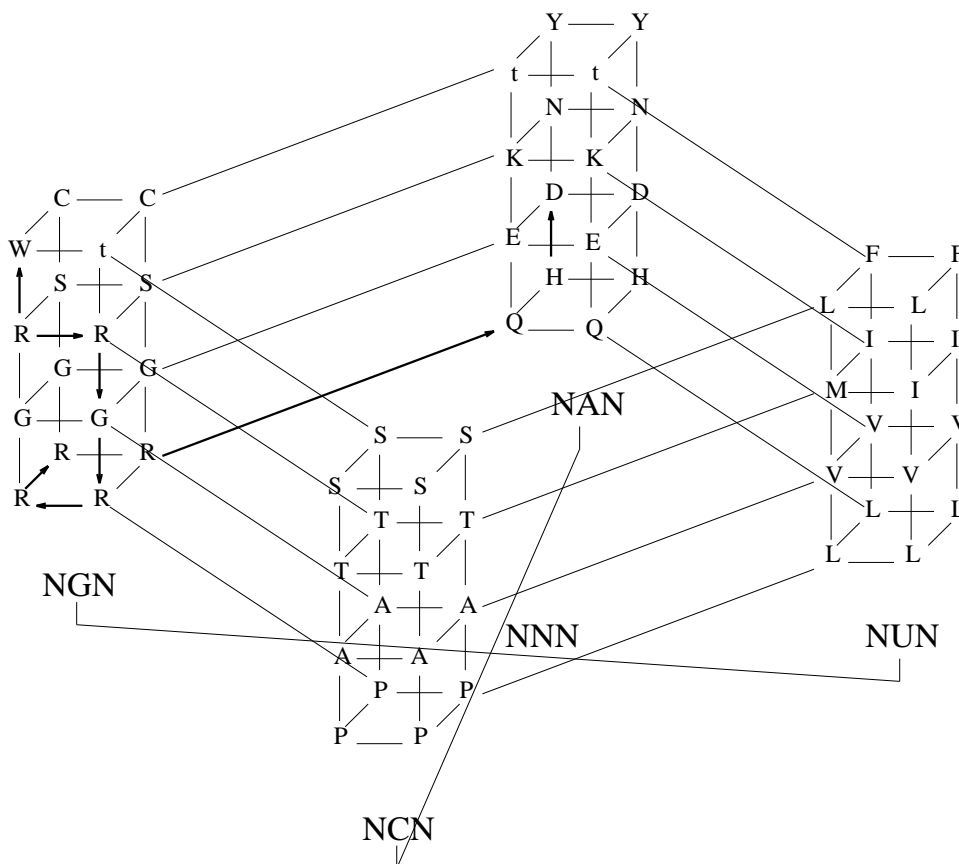


Fig. 2. The six-dimensional hypercube. Each node is labeled with the corresponding amino acid, in the single letter notation, or terminator symbol. For clarity, only some of the links are shown. The cluster of amino acids of the first example discussed in the text and their links are displayed.

The idea to propose a Gray Code representation of the Genetic Code goes back to Swanson [10] where this concept is explained in detail (see also [11]). A great number of different Gray Codes can be associated to the Genetic Code, depending of 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.

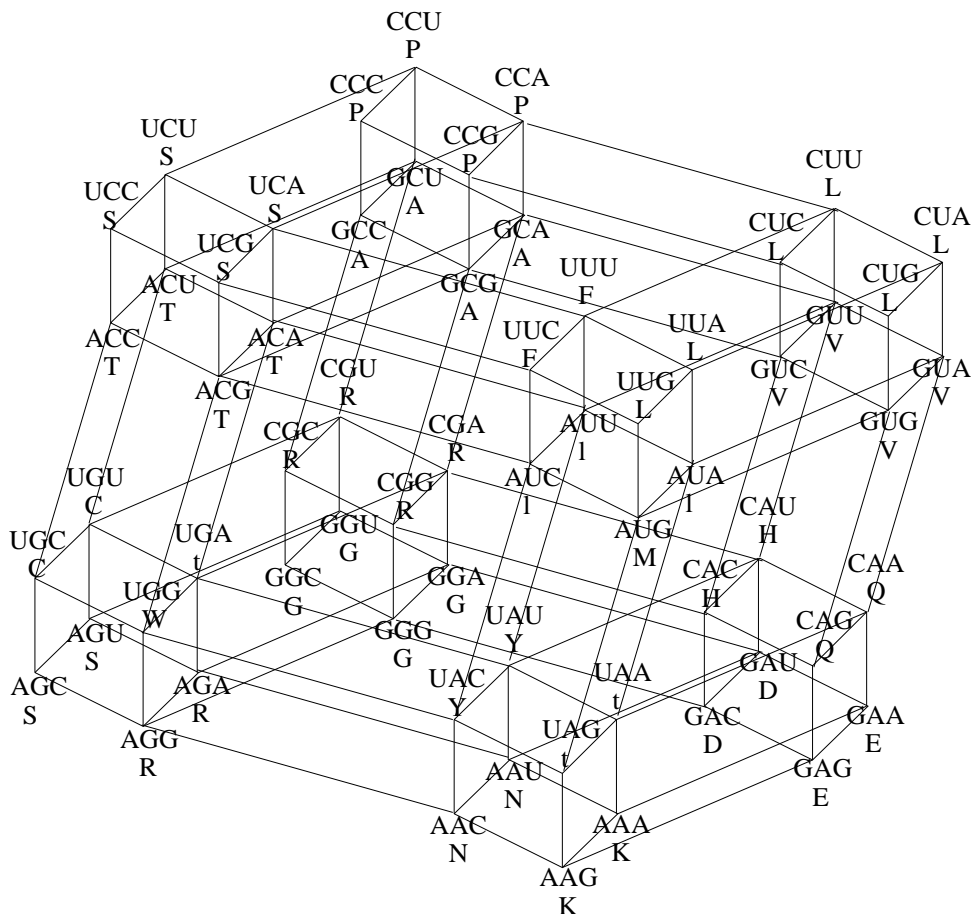


Fig. 3. The hypercube representation of the genetic code. Each node represents a code-word (6-binary vector) of attribute values. However, for clarity of interpretation, the nodes are labeled with the corresponding codons (See Table 1 for the assignment of codons to vectors). The nodes and links mentioned in second example discussed in the text are shown.

4. The Structure of Codon Doublets

This section is more mathematical than the rest of the paper, therefore it is suggested to non-mathematical readers to skip the details. This will not be an obstacle for the understanding of the rest of the paper.

In a pioneering paper Danckwerts and Neubert [12] 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. 4), and their analysis will be extended to the B_2B_3 doublets.

They found the following structure for the set M of B_1B_2 doublets:

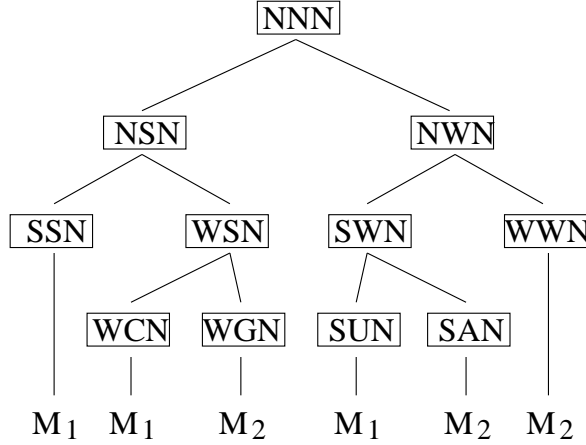


Fig. 4. Decision–tree of codon categories and redundancy distribution. The leaves are the sets of four–fold (M_1) and less than four–fold (M_2) degenerate B_1B_2 doublets.

Starting from AC generate the set:

$$\begin{aligned}
 M_0 &= \{[(1, 1) \cup (\alpha, 1) \cup (\alpha, \beta) \cup (\alpha, \gamma)]AC\} = \{AC, CC, CG, CU\} \\
 M_1 &= [(1, 1) \cup (\beta, 1)]M_0 \quad \text{and} \\
 M_2 &= (\alpha, \alpha)M_1
 \end{aligned}$$

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:

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

Where the base exchange operators (α, β, γ) 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 information² 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 8 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).

² see also [8]

The above results can be simply visualized as a decision-tree (Fig. 4). As can be seen from this figure, the redundancy of a codon is determined only by the H-bond character of B_1 and B_2 : SSN codons (with 6 H-bonds in B_1B_2) belong to M_1 while WVN codons (with 4 H-bonds in B_1B_2) belong to M_2 . However, for codons WSN and SWN (with 5 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 Thryptophan) will require the specification of the six attributes.

From the decision rules obtained from Fig. 4 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 “2-out-of-3” hypothesis of Lagerkvist [8], which refers only to codons in the SSN class.

The sixteen B_1B_2 doublets can be represented as the vertices of a four-dimensional hypercube (Fig. 5). As can be seen from this figure, 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 [13], which considered as basic transformations α and β instead of β and γ as we did. Since the operator α changes two bits we do not consider it as basic.

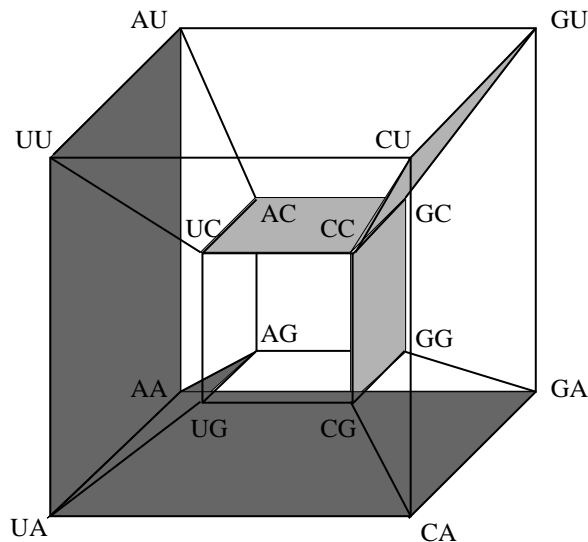


Fig. 5. The four-dimensional hypercube representation of the sets M_1 and M_2 .

Lets consider now the structure of the set M' of B_2B_3 doublets. Exactly as

before, define the following sets:

$$\begin{aligned}
M'_0 &= \{NC\} \\
M'_1 &= [(1, 1) \cup (1, \beta)]M'_0 \quad \text{and} \\
M'_2 &= (\alpha, \alpha)M'_1 \quad (\text{alternatively } M'_1 = (\alpha, \alpha)M'_2)
\end{aligned}$$

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

$$\begin{aligned}
M' &= M'_1 \cup M'_2 \quad \text{can be expressed as} \\
M' &= [(1, 1) \cup (1, \beta)] [(1, 1) \cup (\alpha, \alpha)]M'_0
\end{aligned}$$

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

The sets M'_1 and M'_2 are invariant under the (1, b)–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 [14]. 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 I 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 [14]. Only after the last attribute H_3 was introduced the universal code was obtained with the split of AUR into AUA (I) and AUG (M); and UGR into UGG (W) and UGA (t). It has been speculated that primordial genes could be included in a 0.55–kb open reading frame [15]. The same authors calculated that with two stop codons this open reading frames would have appeared too frequently. From the present view the assignment of UGA to a stop codon was a late event that optimized this frequency (this interpretation differs from the one proposed in [15] and [16] which assume a primordial code with three stop codons). Other deviations of the universal code most likely also occurred in the last stages of the codes evolution.

In the same way as before the sixteen B_2B_3 doublets can be represented as the vertices of a four–dimensional hypercube (fig. 6). The sets M'_1 and M'_2 are also located in compact regions. Codons with B_2B_3 in M'_1 are frequently used in eukaryots. In contrary, codons with B_2B_3 in M'_2 are frequently used in prokaryots. The described structure of the code allows a modulation of the codon–anticodon interaction energy [17].

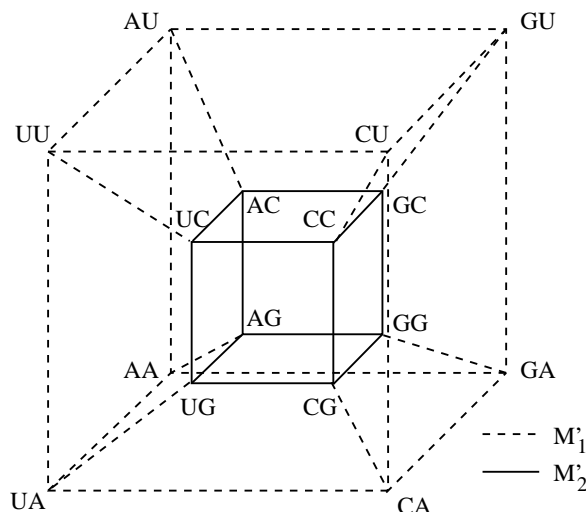


Fig. 6. The corresponding to fig. 5 hypercube of the sets M'_1 and M'_2 . Notice that in both cases each set is located in a compact region.

5. Results

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. In the first example (Fig. 7) we discuss the alignment studied by the method of hierarchical analysis of residue conservation of Livingstone and Barton (Fig. 2 of [18]). 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 [19] we see that, from the properties proposed by Grantham [20] (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. 3) we consider site 33 of the alignment of 67 SH2 domains, Fig 6 of [9]. We can see from Fig. 3 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 (S) accounts for the minor appearance of the small neutral amino acids A, T, P. In a similar way, the variation of the hypervariable region of immunoglobulin kappa light FR1 at position 18 can be explained (Fig. 7. Finally, by looking to the residue frequencies in 226 globins displayed in Table 3 of the paper

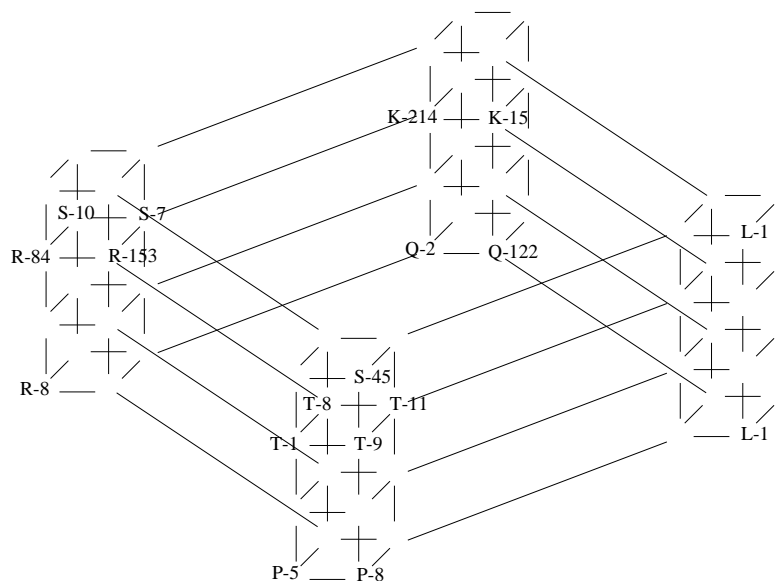


Fig. 7. The amino acid hypercube with the amino acids at position 18 of the variable region of kappa light chain displayed. The number after the amino acid symbol is the number of times the amino acid occurs in the alignment of Kabat et al. (1991): Sequences of proteins of immunological interest, 5th ed. NIH, Bethesda, MD.

by Bashford et al. [21] it is seen 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 present. 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, and the second one with closed trajectories in the hypercube. The corresponding figures are not included because of lack of space.

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 variations found at the variable positions of proteins do not corresponds to random jumps at the codon level, but to well defined

regions of the hypercube.

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