A Minimum Principle in mRNA Editing ?

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Abstract. mRNA editing of sequences of many species is analyzed. The nature of the inserted nucleotides and the position of the insertion sites, once fixed the edited peptide chain, are explained by introducing a minimum principle in the framework of the crystal basis model of the genetic code introduced by the authors.

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1. Introduction

One of the basic dogmas of molecular genetics states that the information contained in DNA flows faithfully, via the mRNA intermediate molecule, into the production of proteins. In 1986 [1], it has been discovered in trypanosoma mitochondria that the information contained in DNA is not always found unmodified in the RNA products. In the following fifteen years, it has been demonstrated that in several organisms (kinetoplastid protozoa, mitochondria or chloroplasts of plants, mammalian cells), some yet unknown biochemical machinery alters the sequence of the final transcription products. This process is called *RNA editing*. For an extensive list of articles on RNA editing, the reader can look at the many web sites on RNA editing [2, 3].

The alteration of the sequence of nucleotides in the RNA occurs after it has been transcribed from DNA but before it is translated into protein. Post-transcriptional modifications have also been observed and interpreted as RNA editing. RNA editing occurs by two distinct mechanisms: 1) *substitution editing*: chemical alteration of individual nucleotides (the equivalent of point mutations), usually C → U. These alterations are catalyzed by proteins that recognize a specific target sequence of nucleotides (much like restriction enzymes). 2) *insertion/deletion editing*: insertion or deletion of nucleotides in the RNA (usually U or C). It is generally believed that these alterations are mediated by guide RNA molecules (gRNA) that base-pair as best they can with the RNA to be edited and serve as a template for the addition

(or removal) of nucleotides in the target [4]. However there is no evidence for the presence of the gRNA for all concerned biological species.

The main features of mRNA editing are:

- the insertion (generally multiple) of U nucleotides or of a single C nucleotide.
- the large majority of the transition involves $C \rightarrow U$. A few cases of transitions $U \rightarrow C$ have also been reported.
- mRNA editing modifies a few percent (0.8 to 5.8 %) of the nucleotides of a specific transcript.
- the mRNA editing appears as a random event, but most of the edited nucleotides occurs at certain hotspots.

As a consequence of the RNA editing, there is a change in the final biosynthesis of amino acids, the most frequent changes being $Pro \rightarrow Ser$, $Ser \rightarrow Leu$, $Ser \rightarrow Phe$. The deep mechanism which causes RNA editing is still unknown. The understanding of the event is complicated: from a thermodynamics point of view a change, i.e. $C \rightarrow U$, takes place if it is favored in the change of enthalpy or entropy, but should this be the case, the change should appear in all the organisms. Moreover from a microscopic (quantum mechanical) point of view, the change should occur in both directions, i.e. $C \leftrightarrow U$. It seems that the primary aim of mRNA editing is the evolution and conservation of protein structures, creating a meaningful coding sequence specific for a particular amino acid sequence.

The purpose of this paper is to propose an effective model to describe the RNA editing. Our model does not explain why, where and in which organisms editing happens, but it gives a framework to understand some specific features of the phenomenon. The paper is organized as follows. In section 2, we analyze the mRNA editing in *Physarum polycephalum*. We first consider this biological species for two reasons: the high statistics of the available data, and the feature of this editing which is mainly characterized by single C insertions, allowing a more detailed and accurate analysis. We show that the existence of preferred sites as well as the nature of the insertions can be understood by requiring the minimization of a suitable function defined on the codon sequence. This function can be defined as we identify each codon by a set of four half-integer labels. In section 3, we then analyze the generally multiple U insertions occurring in kinetoplastid protozoa and we show that also in this case the mRNA editing is understood by a similar minimization procedure. In section 4, we discuss briefly the substitution editing. Finally, we give a few conclusions and highlights for future developments.

2. Insertion Editing by C

The mRNA editing in *Physarum polycephalum*, discovered in 1991 by R. Mahendran, M. Spottswood and D. Miller [5], has been extensively studied and it presents the peculiar feature to be characterized mainly by C insertions. Main feature of the RNA editing in *Physarum polycephalum* is that in about 80 % of the cases the insertion occurs in the third position of the codon, the insertion sites

are non random and in about 68 % of the cases the C is inserted after a purine-pyrimidine dinucleotide. Moreover no rule for the location of the editing sites has been determined, even if the presence of hotspots have been remarked. We have analyzed three published sequences of mRNA editing in portion of the ATP-9 Mitochondrial, of mRNA of cytochromes c and b of *Physarum polycephalum* [5, 6, 7], showing respectively, 54 insertions of a single C, 62 insertions (59 single C, 1 single U) and 40 insertions (31 single C, 6 single U). As a whole we have analyzed 151 single insertions (144 C and 7 U) in three published sequences of *Physarum polycephalum* [5, 6, 7], remarking that the same amino acid chain could have been obtained by insertion of C in a site different from the observed one or by insertion of a nucleotide different from C or U.

In the whole of the analyzed sequences we have remarked (inserted C nucleotides are underlined):

1. the presence of at least 22 alternative insertion sites for C (15 % of the cases, see Table II), which would produce the same final amino acids, so not altering the protein biosynthesis. For example, at the insertion site 9 of [5], the (observed) sequence is ACCCTTA (Thr Leu), while the (unobserved) sequence with alternative insertion site may be ACTCTA.
2. in at least 108 (resp. 98 and 63) of the 144 single C insertions (75 %, resp. 68 % and 44 % of the cases, see Table III), the same final amino acid may have been obtained by a single U (resp. A and G) insertion. Note that in writing Table III, when the insertion site is ambiguous, i.e. when the inserted C is next to another C, sometimes a shift has been performed.

Moreover, we have to consider the two cases GCC UCU $\rightarrow$ GCU ACU – site 16' – and CUU AAA $\rightarrow$ UUA AAR – site 21* – where C insertion is replaced by an A or an R (R = A, G) insertion together with a shift of the insertion site. A similar analysis has been performed for the single U insertions.

This implies two natural questions: 1) why the insertion sites are the observed ones and not the other ones ? 2) why the C insertion is largely preferred ?

In physics when a phenomenon occurs in one fixed way between many possible choices, one assumes that some minimum principle has to be satisfied. The simplest example is the straight path of light (in absence of strong gravitation fields), corresponding to the shortest path between two point in euclidean geometry (the so-called geodesics). Can we think of the existence of a sort of *minimum principle* to explain mRNA editing and/or other process in DNA ? There are several technical and conceptual difficulties in this way of tackling the problem. One should give a mathematical modelisation of RNA and identify the sequence by a possibly discrete set of variables. Defining a topological metric space depending on discrete variables and introducing on it a variation principle is a hard mathematical problem. Moreover we do not have a priori any theoretical guidelines, such as the Hamiltonian and/or Lagrangian formalisms, so we must have some good empirical grounding to begin with. Of course we do not expect biological processes to be deterministic, as it is the case in classical mechanics; so we have to unite minimum

principle, if any, with random nature of the events, like in quantum mechanics. In the present note, as a first step, we look for a simple function which would take the smallest value in the observed configuration of insertion sites and single C insertion, with respect to the configuration with insertion in alternative sites and/or with a single U, G, A insertion.

The starting point for a mathematical modelisation of DNA or mRNA is the crystal basis model of the genetic code [8] where the nucleotides are assigned to the 4-dim irreducible fundamental representation $(1/2, 1/2)$ of $U_{q \rightarrow 0}(sl(2) \oplus sl(2))$ and any sequence of N nucleotides to the N -fold tensor product of $(1/2, 1/2)$ (for codons, see [8] or Table 4 of [9], here reported in Table I for completeness). As a consequence of the model any nucleotide sequence is characterized as an element of a vector space. Therefore, functions can be defined on this space and can be computed on the sequence of codons. Maybe it is worthwhile to emphasize that for the aim of this paper, it is not necessary to understand completely either the mathematical structure of the crystal basis, or the reason to deal with such a sophisticated mathematical structure (see e.g. [8, 9]). The essential point is that any codon is identified by a set of four half-integer labels and functions can be defined on the codons. We make the assumption that the location sites for the insertion of a nucleotide should minimize the following function for the mRNA or cDNA

$$\mathcal{A}_0 = \exp \left[- \sum_k \left(4\alpha_c C_H^k + 4\beta_c C_V^k + 2\gamma_c J_{3,H}^k \right) \right] \quad (1)$$

where the sum in k is over all the codons in the edited sequence, C_H^k (C_V^k) and $J_{3,H}^k$, are the values of the *Casimir* operator and of the third component of the generator of the H - $sl(2)$ (V - $sl(2)$), see [8], in the irreducible representation to which the k -th codon belongs, see Table 1. Let us recall that the value of the Casimir operator on a state in an irreducible representation (IR) labelled by (J_H, J_V) is

$$C_H(J_H, J_V) = J_H(J_H + 1) \quad \text{and} \quad C_V(J_H, J_V) = J_V(J_V + 1) \quad (2)$$

In (1) the simplified assumption that the dependence of $\mathcal{A}_0$ on the irreducible representation to which the codon belongs is given only by the values of the Casimir operators has been made. The parameters $\alpha_c, \beta_c, \gamma_c$ are constants, depending on the biological species.

The minimum of $\mathcal{A}_0$ has to be computed in the whole set of configurations satisfying to the constraints: i) the starting point should be the mtDNA and ii) the final peptide chain should not be modified. It is obvious that the global minimization of expression (1) is ensured if $\mathcal{A}_0$ takes the smallest value locally, i.e. in the neighborhood of each insertion site. The form of the function $\mathcal{A}_0$ is rather arbitrary; one of the reasons of this choice is that the chosen expression is computationally quite easily tractable. If the parameters $\alpha_c, \beta_c, \gamma_c$ are strictly positive with $\gamma_c/6 > \beta_c > \alpha_c$, the minimization of (1) explains the observed configurations in all cases, except for the cases 12, 33, 45 and 41* where there is equality and the cases 18* and 51* where the minimization is not satisfied (see Table II).

Table I. The eukariotic code. The upper label denotes different irreducible representations.

codon	a.a.	J_H	J_V	$J_{3,H}$	$J_{3,V}$	codon	a.a.	J_H	J_V	$J_{3,H}$	$J_{3,V}$
CCC	Pro	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{3}{2}$	UCC	Ser	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{1}{2}$	$\frac{3}{2}$
CCU	Pro	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$\frac{1}{2}$	$\frac{3}{2}$	UCU	Ser	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$-\frac{1}{2}$	$\frac{3}{2}$
CCG	Pro	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$\frac{3}{2}$	$\frac{1}{2}$	UCG	Ser	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$\frac{1}{2}$	$\frac{1}{2}$
CCA	Pro	$(\frac{1}{2})$	$(\frac{1}{2})^1$	$\frac{1}{2}$	$\frac{1}{2}$	UCA	Ser	$(\frac{1}{2})$	$(\frac{1}{2})^1$	$-\frac{1}{2}$	$\frac{1}{2}$
CUC	Leu	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$\frac{1}{2}$	$\frac{3}{2}$	UUC	Phe	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{1}{2}$	$\frac{3}{2}$
CUU	Leu	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$-\frac{1}{2}$	$\frac{3}{2}$	UUU	Phe	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{3}{2}$	$\frac{3}{2}$
CUG	Leu	$(\frac{1}{2})$	$(\frac{1}{2})^3$	$\frac{1}{2}$	$\frac{1}{2}$	UUG	Leu	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$-\frac{1}{2}$	$\frac{1}{2}$
CUA	Leu	$(\frac{1}{2})$	$(\frac{1}{2})^3$	$-\frac{1}{2}$	$\frac{1}{2}$	UUA	Leu	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$-\frac{3}{2}$	$\frac{1}{2}$
CGC	Arg	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$\frac{3}{2}$	$\frac{1}{2}$	UGC	Cys	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$\frac{1}{2}$	$\frac{1}{2}$
CGU	Arg	$(\frac{1}{2})$	$(\frac{1}{2})^2$	$\frac{1}{2}$	$\frac{1}{2}$	UGU	Cys	$(\frac{1}{2})$	$(\frac{1}{2})^2$	$-\frac{1}{2}$	$\frac{1}{2}$
CGG	Arg	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$\frac{3}{2}$	$-\frac{1}{2}$	UGG	Trp	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$\frac{1}{2}$	$-\frac{1}{2}$
CGA	Arg	$(\frac{1}{2})$	$(\frac{1}{2})^2$	$\frac{1}{2}$	$-\frac{1}{2}$	UGA	Ter	$(\frac{1}{2})$	$(\frac{1}{2})^2$	$-\frac{1}{2}$	$-\frac{1}{2}$
CAC	His	$(\frac{1}{2})$	$(\frac{1}{2})^4$	$\frac{1}{2}$	$\frac{1}{2}$	UAC	Tyr	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$-\frac{1}{2}$	$\frac{1}{2}$
CAU	His	$(\frac{1}{2})$	$(\frac{1}{2})^4$	$-\frac{1}{2}$	$\frac{1}{2}$	UAU	Tyr	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$-\frac{3}{2}$	$\frac{1}{2}$
CAG	Gln	$(\frac{1}{2})$	$(\frac{1}{2})^4$	$\frac{1}{2}$	$-\frac{1}{2}$	UAG	Ter	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$-\frac{1}{2}$	$-\frac{1}{2}$
CAA	Gln	$(\frac{1}{2})$	$(\frac{1}{2})^4$	$-\frac{1}{2}$	$-\frac{1}{2}$	UAA	Ter	$(\frac{3}{2})$	$(\frac{1}{2})^2$	$-\frac{3}{2}$	$-\frac{1}{2}$
GCC	Ala	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{1}{2}$	ACC	Thr	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
GCU	Ala	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$\frac{1}{2}$	$\frac{1}{2}$	ACU	Thr	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$-\frac{1}{2}$	$\frac{1}{2}$
GCG	Ala	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$\frac{3}{2}$	$-\frac{1}{2}$	ACG	Thr	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$\frac{1}{2}$	$-\frac{1}{2}$
GCA	Ala	$(\frac{1}{2})$	$(\frac{1}{2})^1$	$\frac{1}{2}$	$-\frac{1}{2}$	ACA	Thr	$(\frac{1}{2})$	$(\frac{1}{2})^1$	$-\frac{1}{2}$	$-\frac{1}{2}$
GUC	Val	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$\frac{1}{2}$	$\frac{1}{2}$	AUC	Ile	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{1}{2}$	$\frac{1}{2}$
GUU	Val	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$-\frac{1}{2}$	$\frac{1}{2}$	AUU	Ile	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{3}{2}$	$\frac{1}{2}$
GUG	Val	$(\frac{1}{2})$	$(\frac{1}{2})^3$	$\frac{1}{2}$	$-\frac{1}{2}$	AUG	Met	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$-\frac{1}{2}$	$-\frac{1}{2}$
GUA	Val	$(\frac{1}{2})$	$(\frac{1}{2})^3$	$-\frac{1}{2}$	$-\frac{1}{2}$	AUA	Ile	$(\frac{3}{2})$	$(\frac{1}{2})^1$	$-\frac{3}{2}$	$-\frac{1}{2}$
GGC	Gly	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{1}{2}$	AGC	Ser	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{1}{2}$	$-\frac{1}{2}$
GGU	Gly	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$\frac{1}{2}$	$-\frac{1}{2}$	AGU	Ser	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$-\frac{1}{2}$	$-\frac{1}{2}$
GGG	Gly	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{3}{2}$	AGG	Arg	$\frac{3}{2}$	$\frac{3}{2}$	$\frac{1}{2}$	$-\frac{3}{2}$
GGA	Gly	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$\frac{1}{2}$	$-\frac{3}{2}$	AGA	Arg	$(\frac{1}{2})$	$(\frac{3}{2})^1$	$-\frac{1}{2}$	$-\frac{3}{2}$
GAC	Asp	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$\frac{1}{2}$	$-\frac{1}{2}$	AAC	Asn	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$
GAU	Asp	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$-\frac{1}{2}$	$-\frac{1}{2}$	AAU	Asn	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{3}{2}$	$-\frac{1}{2}$
GAG	Glu	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$\frac{1}{2}$	$-\frac{3}{2}$	AAG	Lys	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{1}{2}$	$-\frac{3}{2}$
GAA	Glu	$(\frac{1}{2})$	$(\frac{3}{2})^2$	$-\frac{1}{2}$	$-\frac{3}{2}$	AAA	Lys	$\frac{3}{2}$	$\frac{3}{2}$	$-\frac{3}{2}$	$-\frac{3}{2}$

Table II. From the left: the a.a., the C insertion site, the codons coding for the a.a., the dinucleotide preceding C; the shift with respect to the observed site of the alternative insertion site, the new codons, the dinucleotide preceding C in the alternative site. Ref. to fig. 3 of [5], fig. 2 of [6] (with an asterisk *), fig. 2 of [7] (with a prime ').

a.a.	site	codons	dinucl.	shift	codons	dinucl.
Thr, Leu	9, 24, 55*	ACC, UUA	AC	+1	ACU, CUA	CU
Ile, Leu	23, 30*	AUC, UUG	AU	+1	AUU, CUG	UU
Ala, Phe	32	GCC, UUU	GC	+3	GCU, UUC	UU
Val, Phe	33, 45, 41*	GUC, UUU	GU	+3	GUU, UUC	UU
Ser, Arg	34	UCC, AGA	UC	+1	UCA, CGA	CA
Asn, Phe	12	AAU, UUC	UU	-3	AAC, UUU	AA
Ile, Leu	49, 48*, 20'	AUC, UUA	AU	+1	AUU, CUA	UU
Ala, Leu	5'	GCC, UUA	GC	+1	GCU, CUA	CU
Ser, Phe	43*, 13'	UCC, UUU	UC	+3	UCU, UUC	UU
Thr, Arg	3*	ACC, AGA	AC	+1	ACA, CGA	CA
Ser, Leu	18*	AGU, CUG	GU	-1	AGC, UUG	AG
Val, Leu	23*, 40*	GUC, UUA	GU	+1	GUU, CUA	UU
His, Leu	51*	CAU, CUA	AU	-1	CAC, UUA	CA

In order to deal with the remaining cases and to take into account the observed fact that the dinucleotide preceding the insertion site is predominantly a purine-pyrimidine, we add to the exponent of the function $\mathcal{A}_0$ an 'interaction term' which is equivalent to multiply (1) by the function $\mathcal{A}_1$ where

$$\mathcal{A}_1 = \exp \left[- \sum_i \left(4 \omega_{1c} j_{3,V}^{(i)} \cdot j_{3,V}^{(i-1)} - 4 \omega_{2c} j_{3,V}^{(i)} \cdot j_{3,V}^{(i-2)} \right) \right] \quad (3)$$

The sum in i is over the insertion sites and $j_{3,V}^{(i-n)}$ is the value of the third component of the generator of $V-sl(2)$ of the n -th nucleotide preceding the inserted nucleotide C (i.e. $+1/2$ for C, U and $-1/2$ for G, A) and ω_{1c} , ω_{2c} are constants, depending on the biological species. In the case where the insertion site cannot be unambiguously determined, i.e. when the inserted nucleotide is next to a nucleotide of the same type, (3) should be computed in the configuration which minimizes the value of $\mathcal{A}_1$. If $\omega_{1c} > \omega_{2c} > 0$ and $\omega_{1c} > 12\alpha_c$ the minimization of the function $\mathcal{A} = \mathcal{A}_0 \mathcal{A}_1$ explains all the observed positions for C insertions, see Table II. It is reasonable,

Table III. From the left: the a.a., the codon created by C insertion, the alternative codon created by alternative insertion, the site with reference to fig. 3 of [5], fig. 2 of [6] (with an asterisk *), fig. 2 of [7] (with a prime '). Here X = U, A, G and R = A, G.

a.a.	codon	alt. codon	site
Asn	AAC	AAU	35, 4'
Thr	ACC	ACX	5, 7, 9, 10, 21, 24, 26, 36, 3*, 4*, 5*, 12*, 20*, 26* 33*, 35*, 39*, 49*, 50*, 55*, 62*, 15', 39'
Ser	AGC	AGU	1*, 36*, 34'
Ile	AUC	AUU, AUA	1, 4, 13, 15, 17, 18, 20, 23, 38, 46, 49, 50, 51, 6* 7*, 9*, 16*, 17*, 19*, 24*, 27*, 30*, 34*, 38* 48*, 54*, 57*, 58*, 60*, 61*, 20', 32', 36', 37'
His	CAC	CAU	44*
Pro	CCC	CCX	17'
Arg	CGA	AGA	30
Leu	CUA	UUA	31, 40, 8*, 51*, 6'
Leu	CUG	UUG	18*
Leu	CUC	CUX	22
Leu	CUU	UUR	3, 13*, 21*, 47*, 8', 39'
Asp	GAC	GAU	54
Ala	GCC	GCX	25, 27, 29, 32, 37, 10*, 13*, 28*, 53*, 5', 16', 27', 30'
Val	GUC	GUX	2, 6, 11, 14, 33, 42, 45, 23*, 40*, 41*, 56*, 9', 21', 25'
Tyr	UAC	UAU	43
Ser	UCC	UCX	34, 42*, 2', 12', 13'
Phe	UUC	UUU	12, 52, 45*

but not taken into account in (1), to argue that the insertion sites and the nature of the inserted nucleotides also depend on the content of the particular sequence. Moreover $\mathcal{A}$ might be considered as the first terms of a development, next terms involving representations corresponding to more than one codon, the nature of the nucleotides following the insertion site, etc. These further terms may play a role in a more refined analysis.

An analysis of the 7 single U insertions shows that in 6 cases – sites 22*, 10', 18', 22', 24', 26' – (resp. 3 cases – sites 10', 18', 24' –) the replacement $U \rightarrow C$ (resp. $U \rightarrow R$) gives the same amino acid. In 4 of these cases the minimization of Eq. (1) should prefer the insertion of C, giving rise to $UUU \rightarrow UUC$, site 22*; $CUU \rightarrow CUC$, sites 10', 18'; $ACU \rightarrow ACC$, site 24', while in sites 22', 26' UUA is more preferred than CUA. This may explain why the U insertions are so rare compared with the C insertions. Also in this case further terms in $\mathcal{A}$ may help for a more refined analysis of the preferred configuration of the insertions.

3. Insertion Editing by U

The mRNA editing with insertion of U has been observed in particular in a group of parasitic protozoa known as kinetoplastid protozoa. Contrary to the C insertion case where only single nucleotide insertions occurs, the main characteristics of the mRNA editing by U insertion is that the U nucleotides are inserted by blocks. In this way, almost all amino acids can be obtained with a great proportion of Phe and Leu. Many sequences where mRNA editing with U insertion occur can be found in [10] and an extensive list of references on the U insertion editing can be found in [3]. We limit ourselves to cite the first papers on the subject [11, 12, 13, 14]. The table below shows the species and the genes that have been used in our analysis. In this table, COX = cytochrome oxidase, Cyt b = cytochrome b, G = G-rich region, NADH = NADH dehydrogenase, RPS12 = ribosomal protein S12, MURF = maxicircle unidentified reading frame. The number of edited sites is quite large (more than 1000 sites).

species	ATPase6	COX I	COX II	COX III	Cyt b	G3	G4
Crithidia fasciculata	X		X	X	X		
Leishmania tarentolae	X		X	X	X	X	X
Phytomonas serpens	X					X	
Trypanosoma brucei	X			X	X		X
Trypanosoma borreli		X			X		
Trypanosoma cruzi	X		X				

species	MURF2	NADH3	NADH7	NADH8	NADH9	RPS12
Crithidia fasciculata	X		X			X
Leishmania tarentolae	X	X	X	X	X	X
Phytomonas serpens				X		X
Trypanosoma brucei	X	X	X	X	X	X
Trypanosoma borreli						X
Trypanosoma cruzi						

Species and genes used in the U insertion mRNA editing analysis.

Following the same analysis as in the previous section, we make the assumption that the location sites for the insertion of a U nucleotide should minimize the following function for the mRNA:

$$\mathcal{A}'_0 = \exp \left[- \sum_k \left(4\alpha_u C_H^k + 4\beta_u C_V^k + 2\gamma_u J_{3,H}^k \right) \right] \quad (4)$$

When choosing the parameters α_u , β_u , γ_u such that $\alpha_u, \gamma_u < 0$ and $\beta_u > 0$ with $\gamma_u/6 < \alpha_u$, the minimization of (4) explains all the observed configuration, except in the cases $\text{CG}\underline{\text{U}}$ and $\text{GG}\underline{\text{U}}$ where the configurations CGA and CGU on the

one hand and GGA and GGU on the other hand are equivalent (inserted U are underlined). Multiplying Eq. (4) by the corrective term

$$\mathcal{A}'_1 = \exp \left[- \sum_i 4 \omega_{1u} j_{3,V}^{(i)} \cdot j_{3,V}^{(i-1)} \right] \quad (5)$$

with $\omega_{1u} < 0$, the observed configurations become the preferred ones.

It may happen that different U insertions lead to the same configuration of amino-acids (note however that in the U insertion case, this is much less frequent than in the C insertion case, since in the U insertion case, the U nucleotides are inserted by blocks). In the analyzed sequences, we have noticed six such possible alternative configurations:

- in *Leishmania tarentola*, gene NADH8, at edited position 229, one observes the configuration $C_1 = \text{GCU CUA}$, the alternative configuration is $C_2 = \text{GCC UUA}$, and one has $\mathcal{A}_0(C_1) < \mathcal{A}_0(C_2)$.
- in *Phytomonas serpens*, gene NADH8, at edited position 355, one observes the configuration $C_1 = \text{GCA AUU}$, the alternative configuration is $C_2 = \text{GCU AUA}$, and both configurations are equivalent: $\mathcal{A}_0(C_1) = \mathcal{A}_0(C_2)$.
- in *Trypanosoma borreli*, gene cytochrome c oxidase I, at edited position 1375, one observes the configuration $C_1 = \text{GUA AUU}$, the alternative configuration is $C_2 = \text{GUU AUA}$, both configurations are equivalent: $\mathcal{A}_0(C_1) = \mathcal{A}_0(C_2)$.
- in *Trypanosoma brucei*, gene cytochrome oxidase III, at edited position 645, one observes the configuration $C_1 = \text{GCA UUG UUA UUU AUU}$, the alternative configuration is $C_2 = \text{GCU UUA UUG UUU AUA}$, both configurations are equivalent: $\mathcal{A}_0(C_1) = \mathcal{A}_0(C_2)$.
- in *Trypanosoma brucei*, gene NADH7, at edited position 988, one observes the configuration $C_1 = \text{CCG GGU}$, the alternative configuration is $C_2 = \text{CCU GGG}$, and one has $\mathcal{A}_0(C_1) > \mathcal{A}_0(C_2)$. This is a counter-example, however in the configuration C_2 , the nucleotide U is inserted after a C, which is not favored.
- in *Trypanosoma brucei*, gene NADH8, at edited position 251, one observes the configuration $C_1 = \text{UGC CCU}$, the alternative configuration is $C_2 = \text{UGU CCC}$, and one has $\mathcal{A}_0(C_1) > \mathcal{A}_0(C_2)$. This one is also a counter-example.

In the above cases where the insertion sites are not unambiguously determined, multiplying Eq. (4) by the following corrective term

$$\mathcal{A}''_1 = \exp \left[- \sum_i \left(4 \omega_{1u} j_{3,V}^{(i)} \cdot j_{3,V}^{(i-1)} - 4 \omega_{2u} j_{3,V}^{(i)} \cdot j_{3,V}^{(i-2)} \right) \right] \quad (6)$$

with $\omega_{1u} < 0$ and $\omega_{1u} + \omega_{2u} > 0$, the observed configurations become the preferred ones.

In conclusion, the observed U insertions minimize the function $\mathcal{A}' = \mathcal{A}'_0 \mathcal{A}''_1$, except for two cases for which alternative insertion sites exist, where the function $\mathcal{A}'$ takes a lower value, at least in the simplified hypothesis that $\mathcal{A}''_1$ is a perturbative

term to $\mathcal{A}'_0$. It should however be noted that such perturbative term takes into account the nature of the neighbor nucleotides and the experimentally observed bias in the selection of the insertion sites shows an important effect of the neighbors.

4. Substitution Editing by $C \rightarrow U$

Substitution editing of mRNA by $C \rightarrow U$ occurs for example in plant mitochondria and chloroplasts and in the gene apoB in mammals (see web site # 4 in [2]). For our study we have used the COXII gene of the wheat [15]. Similar radical amino acid substitutions in plant COXII sequences have been inferred. Although the statistics is rather poor, we can extract interesting features. In the wheat COXII gene, one observes the following substitutions: CGG $\rightarrow$ UGG (twice), CCU $\rightarrow$ UCU, UCA $\rightarrow$ UUA (twice), UCG $\rightarrow$ UUG, CGU $\rightarrow$ UGU, ACG $\rightarrow$ AUG, so that the corresponding amino acids Trp, Leu, Leu, Cys, Met are correctly coded by the universal code. In [16], the following substitution editing has been observed in several wheat genes (COXII, COXIII, Cob, NAD3, NAD4, RPS12): CGG $\rightarrow$ UGG (seven times), CAC $\rightarrow$ UAC, CAU $\rightarrow$ UAU, UCA $\rightarrow$ UUA, UCG $\rightarrow$ UUG (twice), UCU $\rightarrow$ UUU (three times), CUC $\rightarrow$ UUC, CCG $\rightarrow$ CUG, CCA $\rightarrow$ CUA. In the case of the mammalian gene apoB, the editing depends on the location of the mRNA in the body of the species under consideration (editing in the intestine but no editing in the liver). It is characterized by CAA $\rightarrow$ UAA (Gln $\rightarrow$ Stop codon).

As before, one can easily check that the function $\mathcal{A}'_0$ of Eq. (4) minimizes the configuration corresponding to the substituted nucleotide with respect to the original one.

In [6], three cases of substitution editing are reported in the col gene of Physarum polycephalum. Also in this the function $\mathcal{A}'_0$ is minimized. However, this function differs from the function $\mathcal{A}_0$ Eq. 1 of Physarum.

5. Conclusion

We have shown that the nature of the inserted nucleotides and the position of the insertion site can be explained by introducing a minimum principle in the framework of the crystal basis model of the genetic code introduced in ref. [8]. Indeed, we have made the assumption that, once fixed the final edited peptide chain, the nature and the position of the inserted nucleotide(s), are such to minimize the functions eqs. (1)-(3) or (4), where the numerical real coefficients depend on the biological species, and the operators $C_{H,V}$ and $J_{3,H}$ have to be evaluated on the edited codons using Table I.

Our analysis shows that, in the case of Physarum polycephalum, in 110 of the 114 sites in which the insertion of C or U, and in all the cases where also an insertion of purine can produce the same amino acid, the observed mRNA editing makes use of the nucleotide C or U which does minimize $\mathcal{A} = \mathcal{A}_0 \mathcal{A}_1$. In the case of the U insertion in kinetoplastid protozoa genes, in all the cases but two, the

function $\mathcal{A}'$ is minimized. This last function is also minimized in the case of C $\rightarrow$ U substitution editing.

The form of the function assumed to be minimized has been suggested by simplicity and easiness of computation. For these reasons we have only considered a dependence on the values of the Casimir operator C_H and C_V , although generally there is a degeneracy in the irreducible representations. We have also made the hypothesis that the effects of neighboring nucleotides is weak and limited to the two foregoing ones. As we said previously, we are first of all investigating solid empirical grounds bearing the approach under consideration out, looking then for further mathematical refinements which may give also quantitative information.

We have not considered insertion by nucleotides different from C and U since the statistics is very low. We have assumed that the constants α , β , γ depend on the biological species. However our analysis cannot exclude that indeed they depend only on the type of the inserted nucleotide. It would be interesting to analyze further data on mRNA editing in the analyzed as well as in other biological species to check that the minimum principle is satisfied. Further confirmation of the validity of our hypothesis would provide evidence in favor of the existence of strong physical chemical constraints in the domain generally believed dominated by casual events. The presence of a minimum principle which is indeed an indication of the possible application of variational principle in the field of complex biological systems would be an amazing result.

In conclusion our *effective model* does not explain why and where mRNA editing occurs, but it seems to be able to determine the location sites and the nature of inserted nucleotides, once fixed the amino acid chain.

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References

1. Benne, R., Van den Burg, J., Brakenhoff, J.P., Sloof, P., Van Boom, J.H. and Tromp, M.C.: Major Transcript of the Frameshifted COXII Gene from Trypanosome Mitochondria contains Four Nucleotides that are not Encoded in the DNA, *Cell*. **46** (1986), 819.
2. Some addresses of web sites about RNA editing:
http://www.hysa.org/~jkimball/BiologyPages/R/RNA_Editing.html
<http://nsm1.utdallas.edu/bio/Miller/physarum/physedit.htm>
<http://www.hhmi.ucla.edu/simpson/>
<http://www.rna.ucla.edu/>
3. <http://www.rna.ucla.edu/trypanosome/edrefs.html>
4. Blum, B., Bakalara, N. and Simpson, L.: A Model for RNA Editing in Kinetoplastid Mitochondria: 'Guide' RNA Molecules Transcribed from Maxicircle DNA Provide the Edited Information, *Cell*. **60** (1990), 189.

5. Mahendran, R., Spottswood, M.R. and Miller, D.L.: RNA Editing by Cytidine Insertion in Mitochondria of *Physarum polycephalum*, *Nature* **349** (1991), 434.
6. Gott, J.M., Visomirski, L.M. and Hunter, J.L.: Substitutional and Insertional RNA Editing by the Cytochrome c Oxidase Subunit 1 mRNA of *Physarum polycephalum*, *J. Biol. Chem.* **268** (1993), 25483.
7. Wang, S.S., Mahendran, R. and Miller, D.L.: Editing of Cytochrome b mRNA in *Physarum polycephalum*, *J. Biol. Chem.* **274** (1999), 2725.
8. Frappat, L., Sciarrino, A. and Sorba, P.: A Crystal Base for the Genetic code, *Phys. Lett. A* **250** (1998), 214 (electronic archive physics/9801027).
9. Frappat, L., Sciarrino, A. and Sorba, P.: Crystallizing the Genetic Code, to appear in *J. Biol. Phys* (electronic archive physics/0003037).
10. <http://www.rna.ucla.edu/trypanosome/grnas.html>
11. Shaw, J., Feagin, J., Stuart, K. and Simpson, L.: Editing of Kinetoplastid Mitochondrial mRNAs by Uridine Addition and Deletion Generates Conserved Amino Acid Sequences and AUG Initiation Codons, *Cell* **53** (1988), 401.
12. Feagin, J., Shaw, J., Simpson, L. and Stuart, K.: Creation of AUG Initiation Codons by Addition of Uridine Within Cytochrome b Transcripts of Three Kinetoplastid Species, *Proc. Natl. Acad. Sci. USA* **85** (1988), 539.
13. Feagin, J.E., Abraham, J.M. and Stuart, K.: Extensive Editing of the Cytochrome c Oxidase III Transcript in *Trypanosoma brucei*, *Cell* **53** (1988), 413.
14. Simpson, L. and Shaw, J.: RNA Editing and the Mitochondrial Cryptogenes of Kinetoplastid Protozoa, *Cell* **57** (1989), 355.
15. Covello, P.S. and Gray, M.W.: RNA Editing in Plant Mitochondria, *Nature* **341** (1989), 662.
16. Gualberto, J.M., Lamattina, L., Bonnard, G., Weil, J.H. and Grienemberger, J.M.: RNA Editing in Wheat Mitochondria Results in the Conservation of Protein Sequences, *Nature* **341** (1989), 660.