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The generation of meaningful information in molecular systems

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Summary

The physico-chemical processes occurring inside cells are under the computational control of genetic (DNA) and epigenetic (internal structural) programming. The origin and evolution of genetic information (nucleic acid sequences) is reasonably well understood, but scant attention has been paid to the origin and evolution of the molecular biological interpreters that give phenotypic meaning to the information that is quite faithfully replicated during cellular reproduction. The near universality and age of the mapping from nucleotide triplets to amino acids embedded in the functionality of the protein synthetic machinery speaks to the early development of a system of coding which is still extant in every living organism. We take the origin of genetic coding as a paradigm of the emergence of computation in natural systems, focusing on the requirement that the molecular components of an interpreter be synthesized autocatalytically. Within this context it is seen that interpreters of increasing complexity are generated by series of transitions through stepped dynamic instabilities (non-equilibrium phase transitions). The early phylogeny of the amino acyl-tRNA syntetases is discussed in such terms, leading to the conclusion that the observed optimality of the genetic code is a natural outcome of the processes of self-organisation that produced it.

Introduction

Nucleic acids are generally perceived as sources of encoded information, whereas other copolymers are not, even when they have sequences of comparable complexity. This perception arises on account of the role that nucleic acids, more precisely their sequences, play in biological systems. An organism's DNA, synonymous now with its genome sequence, is often described as its "blueprint" and the DNA acronym is used colloquially to refer to the embedded determinants of the character of any system, biological or not. The closest the blueprint metaphor has ever approached reality in molecular biology is in the work of Gibson *et al.* [1], who substituted a synthetic DNA blueprint for the genome of a *Mycoplasma capricolum* cell, thereby providing the organic structure of the cell with the opportunity to construct a new strain (JCVI-syn1.0) of a related organism, *M. mycoides*. Denucleated human stem cells or DNA-voided cells of *Eschericia coli* are presumably incapable of using the JCVI-syn1.0 blueprint to construct viable structures because there is no appropriate match between the operations executed upon reception of the blueprint and the conventions implicit in the blueprint specifications. The operations carried out by the *M. capricolum* organic structure were efficacious in relation to the manner in which the information in the JCVI-syn1.0 blueprint was specified, just as an architect's blueprint for a building is efficacious when it is framed within conventions used to translate specifications into physical operations of construction. DNA sequences are not meaningful except in relation to the operations performed by molecular systems to which they are presented. DNA sequences have no greater "natural" or "intrinsic" meaning than sequences of coploymers synthesized randomly in a test-tube, except through their physical and historical embedding in biological systems; or in the case of some arbitrary parts of the JCVI-syn1.0 genome, through their relationship with the human social organisation known as "J. Craig Venter Institute" – I refer here to those parts of the sequence which comprise a cryptographic description concerning aspects of that commercial venture's creation of the genome [2,3].

It is the purpose of this paper to contribute to the task of elucidating what it is about nature that provided for the emergence, around 20 Ps in the past, of complex molecular systems that contained nucleic acid sequences that effectively served as self-descriptions, "blueprints", instructions for the systems' construction. The Darwinian theory of evolution is

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of little relevance. Granted, whatever emerged had to "triumph in the struggle for survival", just as one particular mode of confined electromagnetic oscillations is competitively selected when the phenomenon of lasing emerges in an optical cavity. However, selection does not and cannot explain the mode of internal organisation of molecular systems whose operation is essentially *computational*.

What happens in organisms can be considered to be computational in the sense that at the fine- or coarse-gained level at which significantly different states are distinguished, the causes of transitions between such states can be adequately accounted for in terms of relatively small algorithms, with thermodynamics providing a context of "inexorable process" whose control rather than occurrence is the main determinant linking the distinctly biological features of events. The common features of statistical mechanical micro-states that are grouped together into an identifiable biological state are not characterised primarily in terms of their energy distribution, but rather in their sharing some particular (coarser-grained) pattern, or information. The presence of one pattern enables transition to microstates that share some other arbitrary pattern. These are exactly the terms in which England [4] has recently been able to give a detailed thermodynamic calculation of the process corresponding to a food source (f) being used to replicate an extant system (X). However, in this case the informational pattern (e.g., structure of an entire cell, X) is so gross and the algorithm ($X + f \rightarrow 2X$) so trivial that all of the structural and organisational complexity that is so characteristic of biological computation remains obscured, as does the origin of such complexity.

When we talk about biological systems as entities that operate computationally we are saying that the significant changes in the physical state of the heterogeneous mixture of molecules from which they are composed is being controlled by time-varying informatic constraints, which constitute a complex of internal and external boundary conditions on the system dynamics. This is precisely how we describe the changes in the electronic states of digital devices or the macroscopic physical movement of operationally controlled machines. The properties of the material components of the system, which barely change, are a given, as are the law-like mechanics of physico-chemical change, but there is an orderly relationship between states conforming to various patterns of information recognisable at a higher level of organisation. From the point of view of the physical properties of the atomic and molecular components, the orderly transfer and controlling effect of information at the higher level of organisation is completely gratuitous. Thus, changes at the higher level are dictated by differences in identifiable informational control states of internal subsystems and it is therefore correct to describe the causative system dynamics in computational terms. And that is precisely how the language of intentionality or teleology, which is absent from physics and chemistry, creeps into biology. When a biological system is understood in computational terms, it can be said to act, to *do* something: its interaction with the surrounding environment, whether that environment be as simple as the homogeneous local atmosphere or as complex as a diverse ecosystem, is a response to "signs" that corresponding to information which is registered and has a consequential *meaning* in relation to the internal state and dynamics of the system.

Biological codescripts

Some distinguishing features of the control algorithms observed to operate in biological systems compared to other systems in which computations take place were discussed by von Neumann [5]. Biological systems carry a *self-description*, meaningful relative to an *algorithmic interpreter* that the system contains. In the von Neumann model the self-description is instantiated as a readable, copyable "tape" – in real systems, nucleic acid molecules with specific sequences. Von Neumann's algorithmic interpreter is instantiated in a complex, spatially ordered set of mechanical elements that are jointly capable of performing defined operations that directly alter the internal structure/state of their immediate neighbourhood in the system – enzymes, membrane receptors and complexes like ribosomes are molecular biological examples of functional elements involved in intra-cellular algorithmic processes. However, the most important property of von Neumann systems is their reproductive capability, which is the main feature of biological systems that differentiates them from other naturally occurring dissipative structures [6]. The overall system algorithm implicit in the patterned complex of locally defined operations of a von Neumann automaton results eventually in the production of a separated copy of the original system.

In spite of being capable of self-reproduction, and in principle capable of evolving through mutation and selection as a result of changes introduced into the information content of the systems' description tapes during copying of them, von Neumann automata have no conceivable origin except in the design of their construction. It is hard to envisage how anything so complex might spontaneously, even eventually, appear in an randomly arranged (unprimed) dynamic array of the elementary state machines of which the functional systems are comprised; and then sustain existence in the face of disordering noise. Of course von Neumann was not attempting anything so grandiose as an explanation of the origin of life, but it is instructive to reflect on what he omitted from his powerful model as unnecessary or problematic: thermodynamic dissipation (metabolism), statistical mechanical noise and a plausible "molecular" structure-function relationship. We will return to the last of these omissions later in our discussion, only to mention at this point that von Neumann's "world" was made up of connected units of space governed by universal laws of local transformation, in

other words, a "physics" of interaction governing an implicit material world in which computational self-reproduction was instantiated. I will argue presently that the most important and neglected problem in relation to understanding the origin of life is the connection between the mode of genetic information storage (e.g., nucleotide base sequences) and the semi-regular way in which functional phenotypic variations (e.g., in enzymatic catalysis) depend on variations in the structure of system components (e.g., folded proteins). However, it is pertinent first to consider Schrödinger's classic discussion [7] of the physical foundations of living systems.

Schrödinger [7] was the first theoretician explicitly to recognise the role of irreversible, non-equilibrium thermodynamic processes in the generative maintenance of the internal structure of biological systems. In subsequent decades this theme has been developed extensively, originally by Prigogine and coworkers [6,8], to the extent that dynamic nonlinearities are now recognised as necessary for both the original generation and continued sustenance of many meta-scale structures in organisms, as well as being fundamental to systems that fall under the rubric "artificial life". As Schrödinger perceived, irreversible, dissipative processes inside organisms inevitably produce entropy, the effect of which must be compensated if the microscopic order necessary for life is to be maintained. One can explain the compensation in different ways: as either the importation of "negentropy" into the system or the export from the system of the entropy produced as waste. Prigogine extended such considerations by investigating the rate of entropy production as a significant determinant of what is thermodynamically possible in dissipative systems. He was thereby able to characterise unusually ordered states of matter, such as the integrated biochemistry inside living cells, in terms of transitions through instabilities between separate spatio-temporally defined regimes of a system dynamics. Such regimes exist only when there are nonlinear couplings between different internal thermodynamic processes. Thus, the aspect of dissipation absent from von Neumann's self-reproducing systems is now regarded as a central feature of (low entropy) biological systems – without it they could not exist in the real world of energetically and entropically driven processes.

Schrödinger's further and more incisive contribution to theoretical biology was his recognition that no kind of simple macroscopic coupling between thermodynamic processes could account for the detailed manner in which molecular processes are controlled in living cells. His solution was to propose that biological control is essentially computational. He posited the existence of heritable *information*, a copyable *codescript*, effectively specifying "the entire pattern of the individual's future development and of its functioning in the mature state" [7], as the only plausible explanation for the manner in which organisms stave off the disordering effects of the microscropic thermal motion that should otherwise result in their rapid demise. The inheritance of detailed family traits across many generations can only be explained in terms of information, stored in some very compact microscopic form, that can be transmitted through the repeated bottle-neck of the single-cell zygote from which any individual develops. It is necessary that an organism's codescript information be held in a form that is unchanged by the effects of ordinary thermal motion. As we now know, the activation energy for the uncatalysed hydrolysis of DNA far exceeds the typical $\sim 4 \times 10^{-21}$ J available thermally, and the genomic DNA sequence of an organism is accurately copied during every event of cellular reproduction. Thus, Schrödinger's codescript/blueprint idea has become the central precept of molecular genetic reasoning. Were they not under the computational control of a genetic codescript, the inexorable thermodynamic processes occurring within cells would rapidly and comprehensively degrade all orderly systemic biochemistry, as occurs when a cell dies.

Molecular encoding of meaning

The discovery of the genetic code, mRNA and the protein synthetic machinery soon provided a paradigm for thinking about molecular biological processes in computational terms. Furthermore, Crick's Sequence Hypothesis and Central Dogma [9] established an unquestionable consistency between sequence-focussed molecular biology and the neo-Darwinian (selectionist) interpretation of evolution. Later, Eigen [10] described the competitive selection of polymeric sequence information as the guiding principle for understanding the "self-organisation of matter" in biological systems, entrenching the position of DNA sequences, along with their mutation and selection, as the ultimate cause of biological phenomena. The current "systems biology" and bioinformatics approaches to the mounting plethora of molecular biological data represent an attempt to complete the picture by describing *all* of the inter-related mechanisms involved in the production of an individual organism (a single phenotype) from a particular genomic DNA sequence (a single genotype). However, what emerges from such studies is a more confused rather than a clearer picture of not only how genetic information is processed but also the effects of single DNA sequence elements. Thus, DNA is being deposed from its role as the Master Molecule [11] to that of an information-carrying servant of quasi-autonomous molecular biological processes, a growing number of which are recognised to be under mutual epigenetic control.

Faced with the ornate complexity and particularity of molecular biological systems one is tempted to conclude that they will never be amenable to more satisfactory explanations in terms of underlying general principles of construction ("the devil is in the details"); and that explanations of significant evolutionary change will always be limited to "just so" stories ("a chance mutation conferred reproductive advantage in the prevailing historical circumstances"). However this rather pessimistic conclusion reflects our inability to find general rules that specify a direct genotype-to-phenotype mapping for

biological systems, akin to the way the genetic code can ideally be used to discover the structure and properties of proteins using only knowledge of the genetic sequences encoding them. I will argue here that the rules sought cannot be found because they are of a sort that does not exist in nature, not even in the case of genetic coding. According to this interpretation, rather than being fixed in the genetic information that an organism inherits, the genetic code is a complex attractor state of a non-equilibrium statistical mechanical system, the dynamics of which were progressively refined and computationally differentiated largely as a result of a series of symmetry-breaking phase transitions in the prebiotic "phenotype", not as a result of genetic duplication, mutation and selection. Through this reinterpretation of the evolution of genetic coding as phenotypic epigenesis, a first glimpse can be achieved of how imperfect but efficacious computation can spontaneously become instantiated in physico-chemical processes, in spite of the disordering effects of thermal noise and stochastic errors during information replication and code execution. (The term "error" is used with the reservation that it implies that some molecular sequence events are "correct", an attribute that can only be defined in relation to some extant computational rule, the very existence of which we are trying to explain.)

We will now investigate the generative acquisition of "meaning" by polymeric sequence information, such as is found in organisms' DNA, choosing the evolutionary encoding of protein amino acid sequences in nucleic acid sequences as the paradigm of natural processes of this sort. Rather than focusing on the particular assignments of the apparently optimized, near-universal genetic code that finally emerged, we will describe general features of the process of coding evolution and seek to demarcate some prerequisites of coding self-organisation in dynamic molecular systems. These prerequisites amount to informatic relationships between molecular structures and functional properties that are, from a physico-chemical point of view, entirely arbitrary, and which seem so massively coincidental that some theorists have been led to suppose that the code has an extraterrestrial intelligent design [12]. However, what we will see is that similar base triplets are most likely to end up as codons for amino acids with similar molecular properties, not through some process of code optimisation, but simply because each step in the progressive differentiation of coding assignments had to preserve the functionality that had already been established. In fact, information-rich coding is only possible in natural systems that hit upon some operationally encoded definition of "similarity", no matter how rough and ready initially, and then progressively refine it, most likely through transitions over a series of discrete steps.

Polymeric sequence information signifies nothing in itself [13] but it can acquire and have a sustained meaning relative to a system which interprets it and in which it is adequately preserved as a result of ongoing natural selection. The stability criterion of Eigen [10] imposes a limit on the length of such sequences (amount of information) that can be preserved relative to the accuracy of sequence symbol (alphabet letter) copying and the relative fitness of the selected sequence. In extant biological systems the amount of DNA information needed to specify the main components of the machinery of translation (genetic code interpreter) is of order 10^5 bases, if one includes amino acyl-tRNA synthetases (aaRSs), tRNAs, ribosomal RNA and proteins, and initiation, elongation and termination factors. Preservation of this amount of information through cycles of replication requires a complementary base-pair copying accuracy of around $1 - 10^{-5}$ [14] which may be compared with the accuracy of less than $1 - 10^{-4}$ achieved by catalytic subunit of the Qβ replicase, a virally encoded protein about 600 amino acids long which forms a complex with three host proteins to form the functional RNA-replicase complex. More primitive interpreters were no doubt encoded in and correspondingly required the preservation of much less information, but in explaining the emergence of the machinery of translation we face the problem of how it all started from nothing.

Limitations of RNA

The RNA World hypothesis supposes that sophisticated autocatalytic systems based almost entirely on ribozymes were the earliest integrated systems that survived by harnessing environmental energy flows. The *sine qua non* of the RNA World is a putative ribozymal RNA polymerase, which could replicate all components of the system, keeping it alive so to speak. Something like an RNA World may well have preceded the emergence of genetic coding and, as Takeuchi and Hogeweg [15] have shown, it would necessarily have been structured according to "bioinformatic processes" as they were originally defined [16]. From this perspective the RNA World could be considered as a physico-chemical computational system, one that was maintained through the controlled transfer of molecular sequence information. We could speak of such a system having an elementary "code" – complementary Watson-Crick base pairing – and the information for any function would have been stored in the sequence of the ribozyme that performed that function. In fact, it was the capability of RNA to act simultaneously as both an information carrier and a catalyst that led to the RNA World hypothesis, circumventing the need for complex genetic coding in the earliest autocatalytic systems. However, RNA faces a fundamental limitation in respect of its usefulness for the computational control of chemical processes at the sub-nanometre level.

A nucleotide base occupies on average about 0.30 nm^3 compared to an average of about 0.13 nm^3 for the 20 standard amino acids found in proteins. Thus, considering the combinatorial manner in which adjacent spaces in a three dimensional array can be filled with informationally determined choices from 20 (or fewer) distinct small entities rather

than 4 larger entities [17], informationally encoded proteins offer, compared with ribozymes, very much finer computational control over the chemistry of the small regions of space in which individual interatomic bonds are formed or broken. The active site of the hammerhead ribozyme consists of four invariant bases [18]; a ribozyme that aminoacylates RNA has a 3-nucleotide active centre [19]. All else being equal, there is a combinatorial variety of only 4^3 or 4^4 (64 or 256) available from which the maximally active and specific species can be chosen. Taking pyrrolysyl-tRNA synthetase as a typical enzyme there are 6 or 7 residues directly involved in substrate or transition state contact [20], which gives variety in the range 20^6 or 20^7, greater by a factor of some millions than the ribozymal variety within a comparable volume. This comparison does not take into account the fine, energetically constrained, conformational flexibility of local protein structure, the scope of different amino acid chemistries compared to bases and a host of other factors that contribute to the superiority of proteins over nucleic acids as instruments for the specific, differentiating control of chemical processes at the Ångstrom level.

Bowman, Hud and Williams [21] argue from the available evidence that it is very unlikely that the extant system of molecular biological control based on genetic coding derives from a system of similar complexity and sophistication in the RNA World. They are inclined to reject the hypothesis that coded protein synthesis evolved in an RNA World, most components of which later became extinct on account of the takeover of function by more efficient proteinaceous components, including those of the machinery of translation. Growing scepticism concerning the adequacy of an RNA World as the origin of translation [22] has stimulated interest in the manner in which the genetic code could evolve as a result of RNA-protein coevolution [23, 24]. This problem goes far beyond well researched questions concerning mutualism in evolutionary systems and is related to what is sometimes called "Eigen's paradox" (see: [10]), the essence of which is that growth of the amount of sequence information beyond the information threshold set by errors in replication would require a more accurate replicase that could only be encoded in a longer genetic sequence. The need for accurate sequence copying is clear, but there is a similar need for accuracy in gene expression if the phenotypic error catastrophe of Orgel [25,26] is to be obviated. Just as ongoing selection around the attractor state of selection equilibrium is needed to ensure the stability of extant genetic information [10], so is ongoing dynamic self-organisation around the attractor state of coded translation needed to ensure the stability of the error-prone system of protein synthesis [27,28]. And in the same way as we look to the principle of selection to understand the progressive accumulation of genetic information in nucleic acid quasi-species [10], so we must look to dynamically driven processes of self-organisation among mutually autocatalytic protein quasi-species – the statistical proteins of Woese [29] – to understand the prebiotic increase in the specificity of coding that delivered the 61-to-20 codon-to-amino acid code that has been the basis of life since its beginning.

The importance of the 3' acceptor stem of tRNAs in aaRS recognition processes has long been taken to indicate that there was some kind of "operational code" [30] supporting the translation of RNA sequences into protein sequences before the establishment of the strict pairings between tRNA anticodons and amino acids of the genetic code. The term "operational code" is used because this pairing of amino acids to RNA identity elements is thought to have depended on direct "operational" interactions, not the apparently arbitrary arrangement, affected by modern aaRSs, whereby amino acids are paired with RNA identity elements, the anticodon, that are quite remote from the site of tRNA aminoacylation. Carter & Wolfenden [31] demonstrate that an early tRNA acceptor-stem operational code differentiated amino acids quite precisely according to their size, whereas amino acid polarity is the main functional property through which genetic code assignments are recognisably differentiated. That is not to suggest that polarity-based interactions are the direct means whereby the assignments of the genetic code operate. The epoch of the operational code corresponds to a time when the separation of information and function, and thus the computational control of autocatalytic systems, was rather primitive. Evidently, coding of information in RNA sequences migrated from the site of direct interaction between tRNAs and amino acids to the remote anticodon region of tRNAs. This migration from relatively unrefined quasi-analog coding, based on direct local interactions, to digital coding, based on essentially arbitrary (though polarity-ordered) assignments, brought about a new phase in the general, information-dependent, computational control of autocatalytic systems, a prerequisite of Darwinian evolution as a result of genetic variation and selection. Thus, the explanation for the migration of coding to genetic information-based processes is that it offered a *general* and therefore highly efficient solution [17] to the evolutionary problem of finding effectors of chemical events, especially catalysts, that opened new possibilities for the self-organised channelling and integration of thermodynamic flows in the system as a whole.

Encoded proteins

Neither an operational nor a genetic code that mapped alternative nucleotide structures onto amino acid selectivity could provide for the fine, computational control of chemical processes unless there existed a mechanism for the construction of peptides in a manner collinear with nucleotide sequences. Of course ribosomal protein synthesis maintains quite strict sequence collinearity and frame-keeping, but the mechanism of translocation depends on the codon-anticodon interactions of the genetic code, and depends on so many highly specific factors that it gives little clue to its possible origin in the epoch of an operational code. However, operational coding between RNA motifs and amino acids would not

be able to support the informational encoding of useful peptides of any significant length unless there was at least some crude means of peptide synthesis collinear with RNA sequences more than just a few nucleotides long. It is not my intention to propose what such a mechanism may have been, only to suggest that it may have depended on some form of base pairing that brought the aminoacylated termini of RNAs together in such a way as to allow peptide synthesis to be effected sequentially. The reason for thinking that such a process may have initially been achieved "for free", a spinoff from the chemistry of the aminoacylated RNAs, the way information-preserving RNA sequence replication occurs because of complementary base-pairing interactions, is that collinear peptide synthesis has no means of conferring advantage to a system until it actually exists as a mechanism supporting some degree of coded information transfer.

Even if the operational code had its roots in simple stereochemical interactions between amino acids and nucleotide bases, it is notable that the extant remnants from which knowledge of it has been gained are to be found in the highly selective and specific mechanism of catalysis by aaRS proteins. Thus, in relation to nucleic acid-coded protein synthesis, early aaRSs must have been both cause and effect – they evidently catalysed operational code assignments and could only be synthesized as products of those assignments – meaning that the evolution of aaRS specificity and the emergence of the universal genetic code were driven by autocatalysis. It has long been understood that in virtually any system in which peptide synthesis is collinear with RNA sequences, a code can emerge as the outcome of autocatalytic self-organisation [32,28]. And it is abundantly clear that any such code would necessarily be very crude at first on account of both functional and informational constraints: (i) the self-organising transition to a code requires the amplification of functional peptide sequences whose simultaneous initial appearance in the system is improbable; and (ii) the genetic information for the aaRSs supporting a sophisticated code would have to be found through an evolutionary search and, at each stage, would confer no selective advantage in the absence of the species it encoded – hence the need for their initial chance appearance. This is the quintessential chicken-egg problem in molecular evolution and the resolution of it has little to do with Darwinian evolution. Ironically perhaps, its resolution ushers in the era of integrated systems whose further evolution is driven to a great extent by natural selection [33].

The main findings concerning the dynamics of coding self-organisation – the improbability of the appearance of the reflexive sequence information; the general need for a stepwise process; the essential role of spatio-temporal ordering in the emergence of genetic coding – have recently been discussed elsewhere [13]. Here we will consider other aspects of coding evolution more directly concerned with the emergence of the computational control of biochemical processes and the connection with the evolution of the aaRSs: (i) the amino acid sequence dependence of aaRS specificity; (ii) quasi-species phylogeny of aaRS phenotypes; and (iii) the evolution of metabolism.

Sequence dependence of protein function

An enzyme's substrate selectivity is never perfect, even when its amino acid sequence has apparently been optimized over aeons of evolution. This is especially relevant to the coding functionality of the aaRSs, which have to differentiate between potentially similar amino acids as well as tRNAs. If the recognition functions of aaRSs were sensitive to structure to the extent that single amino acid substitutions could leave the enzymes' catalytic capability intact but change which amino acid or tRNA was recognised, then a single random assignment error in a cell could produce a rogue assignment activity that corrupted the operation of the code and led to proteome collapse through an error catastrophe of the type envisaged by Orgel [25]. Thus, the structure-function mapping for the aaRSs, that is, the pattern of the embedding of codon-to-amino acid assignment functions in protein sequence space, an informational pattern which is essentially fixed by the laws of physics and chemistry, is a central determinant of stability of information processing in molecular biological systems.

On the one hand, there is the arcane problem of the potential ambiguity of the "reflexive genetic information", that is, information which specifies, according to a set of codon-to-amino acid assignments corresponding to a code, the protein sequences of the aaRSs which catalyse the relevant set of coding assignments. Due to peculiarities of the embedding pattern of assignment functions in the peptide sequence, sets of assignments for alternative codes can require the same set of genetic sequences but give them an alternative interpretation. This is especially possible for embeddings in low dimensional sequence spaces [34,35]. While this problem has probably been of little practical consequence in the evolution of genetic coding, it illustrates that molecular coding is only possible in a world whose physics throws up entities whose functional properties are related by a particular sort of computational relationship between their structures; and it may pose difficulties for the design of coded information-processing systems employing molecules much smaller than polypeptides and corresponding polynucleotides, that is, simple molecules with relatively few potentially functional features.

On the other hand, the general regularity of functional chemistry underpins the possibility of molecular evolution: molecules with very similar structures tend to have similar chemical properties, and without such regularity there would not be local gradients of fitness in genetic sequence space across which advantageous molecular adaptations could be

optimized. If fitness varied randomly across the entire range of possible point mutations there would be no pathway for progressive systemic improvements through the accumulation of advantageous mutations ijn individual genes. It is in relation to this line of reasoning that it has often been claimed that the genetic code is optimised: a point mutation causing an amino acid substitution in a protein is most likely to specify one that has properties (size, shape, hydrophobicity, polarity, charge, etc.) that are similar to those of the amino acid in the wildtype protein sequence [36]. However, if we construe the argument in terms of the *origin* of the code's optimality, that is, in the role that amino acids in aaRSs sequences play in those proteins' amino acid recognition functions, then it takes on a quite different aspect, as has been pointed out by Guimarães [37,38].

What other sort of outcome, other than an "optimal" one, should we expect from the evolution of the aaRSs, a process in which the functional effects of the available range of specifiable variation (choice of amino acid at a point in a protein sequence) progressively determines how that variation is to be specified? How could some completely unordered, information-dense representation of amino acid functionality, the rules of some completely arbitrary code that would later be optimised through variation and selection among possible sets of rules, have suddenly popped into being? Rather, for any rule (regularity in the operational effect of a "codon") to come into being, no matter how crude it might be at the outset, it had to provide for the representation, in genetic symbolic form, of selectable functional variation. Furthermore, it had to do so in a manner that provided for adaptive selection of amino acid recognition – a process determined by the very functionality to be encoded. On this basis I would argue that no code could evolve selectively unless the symbols (codons) in which it represented elemental functional variety provided an orderly map of that variety. The regularities of our own biological genetic code indicate the effective dimensions in which the topology of amino acid functionality is represented [36,39].

Emergence of binary coding

It is fundamental to molecular biology that aaRS production is a collectively autocatalytic biochemical process – to a first approximation all of the aaRSs are required for the production of any one of them. Standard phylogenetic analysis indicates separate universal common ancestries of all extant Class I and II aaRSs, suggesting an original binary code operated by two well-differentiated urzymes that catalysed different examples of the same generic reaction: aminoacylation of RNA. For the pair to be mutually autocatalytic they must have been of adequately different construction to have maintained disjoint identities, given a certain range of assignment functions catalysed by extant variants of each; otherwise competition would have led to the demise of one of them (a standard result concerning the hurdle to evolutionary cooperation). There is an attractive economy in the Rodin-Ohno hypothesis [40] according to which the urzyme pair's catalytic functions were encoded by complementary strands of a single gene, possibly of length fewer than 150 nucleotides (assuming triplet codons), of which only every third base would need to be specified if the antiparallel frames of translation were phase-matched, a disposition for which there is still evidence [41].

Let us now concern ourselves with how a pair of Class I and II aaRS urzymes may have come to function in a mutually autocatalytic fashion. If there were a prior ribozymal-based coding system which the initial pair of protein aaRSs "took over", it is hard to envisage how urzyme cooperation would have held at bay competition between them – an extant ribozyme rather than the poorly-defined protein partner could compensate for coding deficiencies of one of the urzymes. For takeover through natural selection, the amino acid-based selectivity of the original urzymes would have to encompass the nucleotide-based selectivity principle of the ribozymal coding system. However, if the cooperation (mutual autocatalysis) of the urzymal pair was the only pathway to the production of either of them, as envisaged in any Rodin-Ohno-like scenario, then there is plausibility to the entrenchment of two quite distinct classes of coding-assignment proteins with different structures, something that occurred at the very beginning of protein-catalysed coding.

The manner in which Class I and II aaRS urzymes employed amino acid functionalities to separate them into two broad classes would have constituted the very first step in the bootstrapping of a generative grammar for protein-based catalysis; and the subsequent phylogeny of the aaRSs would represent the stepwise refinement of the grammar whereby the placement of amino acids in proteins was brought under ever more precise computational control, based on the functional effect of those placements. The self-organising transition to a binary code from random protein synthesis, roughly collinear with extant RNA sequences whose replication was peptide assisted [28,42,43], would have created in the space of codons a very coarse-grain, one-bit map of amino acid functionality in proteins. There is no necessity in that first bit of codon information being immediately associated with any recognition decision as clean and simple as a Gray-code choice [39], for example, between R or Y nucleotides at a particular position in triplet codons: there is a very large number of ways of dividing 64 codons into two approximately equal groups. In the event, operational factors, as yet under little informational control, would have been the main influence in determining how the bit was represented. But as the code became refined, its map of amino acid functionality in proteins would have had to become quite orderly.

It is salient to consider the effects of a loose, binary genetic code, given that the number of different amino acids present at various relative concentrations in the prebiotic environment was no doubt greater than two. Ignoring for the moment the presumed poor control of initiation, frame-keeping and termination, the "translation" of any extant RNA sequence could be expected only to show selectivity between two broad (and not necessarily entirely disjoint) classes of amino acids at any position in the resulting peptide sequence, producing a broad "quasi-species" of "statistical proteins" to use the terminologies of Eigen [10] and Woese [27].

The orderliness of chemistry tells us that the members of a protein quasi-species will have a distribution of related functionalities. In the case of aaRS quasi-species, that distribution would correspond to a range of amino acids (and codons) recognised [44,21], so it is no surprise that the first separation of aaRS functionalities depended on two completely different ways (Class I and II) of using arrangements of amino acids in folded peptides to attach an amino acid to an RNA: one method worked for one set of amino acids and associated codons and the other worked for a distinguishably different set of amino acids and corresponding codons. And it is equally unsurprising that the first code of correspondence between amino acids and "codons", however they were defined at that stage, should have been achieved through direct "operational" association, close to the site of catalysis, only later to achieve the appearance of arbitrary "symbolic" matchings more characteristic of computational control.

Quasi-species symmetry breaking

A second whole bit of the coding map would not have been achieved until the Class I and II aaRSs had each split into two subclasses (if that was the order of phylogenetic events) to give four distinguishable subsets of amino acids. The Rodin-Ohno hypothesis provides a natural pathway for this to be achieved in a single step [41] comprising coupled symmetry-breaking events in the protein and RNA populations. It is important to understand that such a process does not correspond to gene duplication followed by selection. It is a dynamically driven process of self-organisation of the sort envisaged to have produced the first binary-code coupling between peptide and RNA populations [42]. When there is only a binary code operating there is no pressure for the selection for the subset of genes that encode machinery for another bit of codon information; and the full variety, across all potential alphabet letters in the as yet non-coding positions (or nucleotide choices), would be accessible within the imperfectly replicating quasi-species of coexisting aaRS-encoding RNA sequences. That means in turn that the individual sequences that specifically catalysed the more refined assignments of a finer grained, two-bit code were just as likely to be present (albeit at diminishingly low concentration) as any other similarly partial random sequence, the vast majority of which would probably display insignificant catalytic activity [28,45].

Thus, the original process of symmetry-breaking self-organisation from a random population of peptides to two, much less diverse, mutually autocatalytic subpopulations with distinguishable binary-code assignment functions, sets up an opportunity for the same process to be repeated. The process would occur when the chance appearance (in the replicating RNA quasi-species) of genes for ternary or quarternary coding was coincident, within some sufficiently confined locale, with the chance appearance of particular members of the protein quasi-species that translate those genes as specifications of themselves according to the rules of the more advanced code. The chance presence of particular proteins with more specific functionality creates a selective advantage for the genes encoding them, pushing the system across the stochastic threshold for the transition to a refined code. This mechanism is likely to have been dominated the early "pre-Darwinian" stages of code development [33], before the main driving force of evolution became competition between well-contained genotype-phenotype entities. During this early phase of molecular evolution, whether because system encapsulation was poor or the rate of horizontal gene transfer was high, the disordering effect of molecular movement had to be overcome to associate genotypic and functional phenotypic molecular forms in the same neighbourhood through mechanisms such as Turing reaction-diffusion coupling [42,15].

Darwinian evolution would not occur until RNA replication and translation had become reasonably accurate, the dynamic sampling within potential quasi-species had become slow and genotypes and phenotypes were collocated. The inefficiency of quasi-species sampling would then make the stochastic initiation of a self-organising transition to an expanded code prohibitively improbable. Thus, the last steps in the development of the code may have occurred by the Darwinian "mutation first" mechanism of gene duplication and selection, as for example a pre-tryptophan code appears to have generated a Tyr-aaRS variant that recognised tryptophan [46]. However, such events can still be regarded as self-organising coding transitions in which the informational specification of *all* of the aaRS functionalities is refined, setting in motion a new process of whole-group, code-wide optimization. The generic process of code expansion could therefore be expected to occur in a stepwise manner until the map of amino acid functionality in codon space was refined to the limit of resolution achievable through protein construction. Later steps in this direction would be limited only by the sustained availability of an amino acid with properties that could improve the code's map of the role of amino acids in aaRS recognition functions. Ilardo *et al*. [47] have provided a convincing demonstration that the universal genetic code indeed corresponds to the achievement of the limit of coding resolution.

Concluding remarks

There is not space in this article to explore the essential link between the refinement of the genetic code and the progressively computational control of metabolic processes. Suffice to say, control and development of the processes that produced the building blocks (amino acids) of the emerging agents of chemical control – protein enzymes with their capacity for subtle allosteric effects – must have been a determinative factor in the autocatalytic harnessing of the free energy resources and fluxes that are the physico-chemical currency of life. Thus, the coevolution theory of code evolution [48,49], especially as it has been expanded by Di Giulio [50,51], supplies the last foundation stone in the picture of coding evolution as an autocatalytically driven process of information-processing self-organisation. If amino acids were originally metabolised as RNA-acylated species then the circumstances already existed for the incorporation into the code of a downstream metabolite of an extant member of the coding set through specialisation of the corresponding aaRS's amino acid- and codon-recognition functions, leading to the compact representation of the coding map constructed by Wong [48]. Thus, we should expect ultimately to uncover intimate links between the functionality of the aaRSs and the enzymes that catalyse the fundamental processes of cellular energy metabolism: glycolysis and the Krebs cycle.

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