# HYPERCOMPLEXITY

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## ABSTRACT

What is biological complexity? How many sorts exist? Are there levels of complexity? How are they related to one another? How is complexity related to the emergence of new phenotypes? To try to get to grips with these questions, we consider the archetype of a complex biological system, *Escherichia coli*. We take the position that *E. coli* has been selected to survive adverse conditions and to grow in favourable ones and that many other complex systems undergo similar selection. We invoke the concept of *hyperstructures* which constitute a level of organisation intermediate between macromolecules and cells. We also invoke a new concept, *competitive coherence*, to describe how phenotypes are created by a competition between maintaining a consistent story over time and creating a response that is coherent with respect to both internal and external conditions. We suggest how these concepts lead to parameters suitable for describing the rich form of complexity termed *hypercomplexity* and we propose a relationship between competitive coherence and emergence.

## 1. INTRODUCTION

Many systems are called 'complex' and might be put into different classes of complexity if the criteria to use were clear. It seems evident that the complexity of biological organisations differs from that of inanimate systems such as the weather but the exact nature of the difference is less evident. One of the characteristics of many complex systems is the phenomenon of emergence in which properties of the system emerge that cannot be readily predicted from a knowledge of the constituents of the system. Its very unpredictability, however, makes emergence hard to work with and model. Although a few other terms such as non-linearity and strange attractors are employed in the field of complex studies, the vocabulary available to describe complex systems is still rather limited and, to begin a taxonomy of complex systems, it would be useful to have an idea of the parameters needed to capture the essence of complex biological organisations.

The model bacterium, *Escherichia coli*, is one of the best-understood of all organisms. It might therefore be expected that the process whereby this 'simple' bacterium divides into two bacteria would be thoroughly understood. This is not the case, probably because bacterial division is dependent on the interplay between many different factors. Division

 

is therefore one example of just how complex a biological process can be and of why a new term, *hypercomplexity*, might be useful. The case can also be made that *E. coli* contains a level of organisation intermediate between macromolecules, such as genes and proteins, and the cell itself: this is the level of *hyperstructures* (Amar *et al*., 2002). Other biological organisations also have intermediate levels and, to take account of hierarchical complexity, the vocabulary of complexity should include levels as a parameter. It could be argued that virtually all biological organisations, including social ones, have to undergo the vicissitudes of a fickle environment. Hence, additional parameters to characterise, and even quantify, hypercomplexity, might be derived based on the essence of organisations subject to selection for growth in good conditions and survival in bad ones. This essence includes the existence of quasi-equilibrium and non-equilibrium structures.

Other parameters, we contend, can be based on the process of *competitive coherence* which underlies the operation of many biological organisations and which can be used to describe the way that a key subset of constituents are chosen to determine the behaviour of an organisation at a particular level (Norris, 1998). This choice results from a competition between the need of the organisation to behave in (1) a consistent way over time so as to maintain historical continuity via the *status quo* and (2) a coherent way at a particular time that makes sense in terms of both internal and environmental conditions and that is highly adaptive. This brings us to a vision of biological organisations orbiting around two pairs of attractors. The first pair is the quasi-equilibrium versus non-equilibrium pair or, for example survival versus growth, spore versus growing cell. The second pair is the continuity versus coherence pair or the history versus the present. Here, we flesh out this vision, we propose candidate parameters for hypercomplexity and, finally, we use competitive coherence to propose that the phenomenon of emergence corresponds to an active subset of constituents having an unexpected coherence with strong selective advantages.

## 2. BIOLOGICAL AXES

The idea that organisms evolve to try to satisfy two opposing constraints is familiar to biologists. Modelling organisms as Boolean networks of genes, for example, has underpinned the idea that organisms evolve to and along the "edge of chaos" between a frozen regime in which each state is the same as the preceding one (and the genetic network only gives one phenotype) and a chaotic regime in which different states succeed one another in endless state cycles (so there are too many phenotypes for any genotype-phenotype relationship to be selectable) (Kauffman, 1996; Langton, 1990). We suggest the selection space for biological organisations has other dimensions. The first dimension is the continuity/coherence axis. In this context, synonyms for continuity are the *status quo* effect and the importance of respecting the organisation's history: synonyms for coherence are internal consistency, coordination and the importance of taking into account the present environment. Somehow the organisation must maintain both the continuity of its composition and its coherence so as to have phenotypes that are consistent both *over time* with one another and at *the present time* with the environment. Failure to achieve such consistency is disadvantageous and, in a competitive world, punishable by extinction.

The second dimension is the quasi-equilibrium/non-equilibrium axis. In this context, synonyms for quasi-equilibrium are static, redundant and robust; quasi-equilibrium structures are about surviving in a difficult environment by becoming independent of the environment and abandoning growth. Synonyms for non-equilibrium are dynamic,

efficient and fragile; non-equilibrium structures are about growing in a favourable environment and therefore becoming vulnerable. Organisations must develop strategies to survive and flourish in a fluctuating environment that presents both opportunities and dangers. Successful biological organisations manage to convert quasi-equilibrium structures into non-equilibrium ones and *vice versa*. Indeed, this ability to balance the ratio of such structures might constitute the basis for another definition of life.

## 3. THE CONCEPT OF COMPETITIVE COHERENCE

To understand the concept of competitive coherence, consider a simple organisation modelled in the following way:

1. the organisation contains many individuals in which these individuals compete for membership of a smaller group, the *Active* set, that decides the behaviour of the organisation.
2. the organisation moves through a series of behavioural states defined by the composition (in terms of individuals) of the *Active* set.
3. membership of the *Active* set in a particular state is decided on the basis of one type of connectivity between the individuals. Each individual $i$ of the current state has connections $C$ to the other individuals, $j$, in the organisation.
4. the competition between individuals to be included in a developing, new state of the *Active* set is based on the frequencies of two groups of connections, *Next* and *Now*, between individuals.
5. the memory of the organisation in terms of the *Next* group of connections is obtained from a long series of states of the *Active* set by recording the connections between an individual present in one state of the *Active* set and those individuals present in the following state of this set. By summing the *Next* connections for each member ($\Sigma^{NEXT}C_{i,j}$) of the current state of the *Active* set (where $i$ is a member of the *Active* set and $j$ an individual in the total set of individuals), the entire set of possible members of the next state may be ranked in order of their degree of connectivity to the set of members actually present in the current state. The individuals with the highest connectivities can then be selected to participate in the new state. This ensures a strong relationship between the members of successive states and hence a continuity. Moreover, members of the present state are likely to be reselected by this process hence its *status quo* nature.

Once a few individuals have been selected to participate in the future state, the *Now* process starts to play a role. The *Now* group of connections is obtained from the connections between individuals that are present together in the same state ($\Sigma^{NOW}C_{i,j}$), i.e. that are members at the same time. Each member of the new, as yet incomplete, state is examined for connections to other candidate members with which it is regularly present in a state and, by summing the connections for all these new individuals, the members of the entire set of individuals can again be ranked, this time in order of their degree of *Now* connectivity. This gives an internal coherence to the *Active* set of members actually chosen to be present together.

Initially, the *Next* process dominates selection for the state of the *Active* set with those present in the current state 'deciding' on some of those that are to participate in the next state. However, there may be an important input from the environment that forces inclusion of certain individuals in the developing new state. Once a few individuals

have been chosen to be members via the *Next* process and via environmental stimuli, the relationship between individuals that can cooperate, the *Now* process, plays an increasing role and the composition of the developing state becomes a competition on the basis of connectivities between the two processes. This means that the composition of the *Active* set is selected so as to be consistent over successive states and to be coherent with respect to the particular state.

In this brief explanation of competitive coherence within a simplified organisation, we have considered that there is only one type of connection between the individuals and hence generating the single *Active* set on the basis of these connections is straightforward. Such an organisation might be an example of a complex system. In many biological organisations, however, there are many types of connection and hence many possibilities for forming the *Active* set according to selection for these different types of connection. The interesting problem here is how to do this. Organisations that have solved this problem, such as bacteria, are *hypercomplex*. One extreme solution is to have an *Active set* for each type of connection and then to have a second level of competitive coherence between the *Active* sets themselves. In essence, each *Active* set becomes an object in its own right and a second round of competitive coherence is performed on these objects using the connections between them; this corresponds to a change of level. A less extreme solution is to have a meta-process that can integrate the different sorts of connectivity competing for influence of the composition of the *Active* set. Possible candidates for such an integrating process include ion condensation onto charged polymers (Ripoll *et al*., 2004) and the dynamics of water (Wiggins, 1990). For example, the integrating process, which reflects the synergy between the types of connection, leads to the formation of a structure; this structure is then the physical manifestation of the *Active* set. It is even possible that the integrating process is space. Members of the *Active* set need space to live, move and have their being. They need space to get together and do their thing. Many of the types of connectivity that play a role in selection for the *Active* set affect the occupancy of space. We illustrate these ideas below.

## 4. CELL DIVISION IN BACTERIA AS AN EXAMPLE OF HYPERCOMPLEXITY

The process of division in the model bacterium *Escherichia coli*, whereby a parental cell divides at its middle to give two daughters, is one, arbitrary, example – of many possible – of how a complex explanation is invoked in biology when a hypercomplex explanation is required. A popular model for division site selection is a reaction-diffusion Turing-type mechanism based on the Min proteins which when defective lead to misplaced division and minicell production and which can oscillate from pole to pole to influence the key division protein, the tubulin-like FtsZ (Meinhardt and de Boer, 2001). The trouble with this explanation is that cell division entails much more than Min oscillation within a relatively unstructured cell. To begin to appreciate the complexity of division, we need to take on board the following. At the level of structures, the chromosomes appear to play a role in inhibiting division around but not between them via the phenomenon of 'nucleoid occlusion' which we have recently tried to explain in terms of the dynamics of membrane domains and FtsZ (Norris *et al*., 2004b), indeed, a very early stage in division site selection involves the formation of membrane domains around the chromosome and at the cell's equator (Fishov and Woldringh, 1999; Mileykovskaya and Dowhan, 2005);

also central to division are the structural dynamics of protofilaments of FtsZ and its relocation during the cell cycle to the equator – and possibly its mechanical action there (for references see (Norris *et al*., 2004b)). Such mechanical action is probably accompanied by changes in membrane composition, packing and curvature at the developing division site (Norris *et al*., 2002b). At the level of ions, a calcium flux occurs at the time of cell division that may also play a role in the dynamics of membranes and FtsZ (Norris, 1989) perhaps via condensation and decondensation on linear charged polymers such as FtsZ filaments (Ripoll *et al*., 2004). A number of other organising processes might also be invoked (see (Norris *et al*., 2004a)).

There is much more to division than this though. A cell can be considered an autocatalytic network and it has been proposed that a major function of cell division is to partition different autocatalytic networks into separate cells (Segre *et al*., 2000); the implication is that to time and position division the growing cell needs a sensor of its network status; this would square with the notion of a metabolic sensor based either on putative interactions between metabolic enzymes and FtsZ or on the density of transertion (coupled transcription-translation-insertion of nascent protein into membrane) structures or on the relative abundance of different water structures. Finally, division is the end result of a state cycle of phenotypes which raises the question of the relationship of this cycle to division.

Insofar as division results from several organising processes rather than from a single organising process, we consider it an example of hypercomplexity rather than complexity. In which case, it may be interesting to analyse it in terms of the parameters of competitive coherence where each organising process corresponds for example to an *Active* set (see below). This would entail separate *Active* sets each dealing with connections between macromolecules based on either calcium-binding or lipid affinities or polymerisation or preferences for oscillating water structures etc. Modelling the operation of more than one *Active* set is not trivial but, that said, certain of the problems associated with operating a system with more than one *Active* set disappear if a multi-level approach is adopted.

## 5. HYPERSTRUCTURES IN BACTERIA

Recently, the existence of a level of organisation mid-way between genes/proteins and whole cells has been proposed. This is the level of hyperstructures. A hyperstructure is an extended assembly of diverse molecules and macromolecules (genes, mRNAs, proteins, ions, lipids etc.) that is associated with at least one function (Amar *et al*., 2002). The concept of hyperstructure embraces more than what is usually meant by a "supramolecular assembly" or a "molecular machine" or even a "module" (Alberts, 1998; Hartwell *et al*., 1999). Hyperstructures come in two flavours, non-equilibrium and quasi-equilibrium. A non-equilibrium hyperstructure is assembled into a large, spatially distinct structure to perform a function and is disassembled, wholly or partially, when no longer required. Its continued existence depends on its consumption of energy. Consider, for example, the structure formed by the coupled processes of transcription and translation, which consume energy in the form of ATP or GTP hydrolysis, during full induction of the *lac* operon in *E. coli* (Kennell and Riezman, 1977); one type of lactose hyperstructure would comprise the *lacZYA* genes (of which there may be more than one set if there is more than one chromosome), the RNA polymerases transcribing these genes, the nascent mRNA, the ribosomes translating this mRNA, and the nascent proteins – including the

permease being inserted into the membrane. The hyperstructure may also comprise any lipids for which the permease has an affinity, as well as inorganic ions such as calcium, polyamines, polyphosphates and other molecules. In addition, there is a second type of putative hyperstructure – that may even be intimately associated with the first – that comprises the fully synthesized proteins in the process of functioning (Norris *et al*., 1999); the idea here is that enzymes in a pathway acquire an affinity for one another *because* they are functioning.

In contrast, a quasi-equilibrium hyperstructure does not dissociate in the absence of a source of energy. Consider again the lactose operon but this time in the repressed state. This involves the tetrameric repressors binding to both the operator and neighbouring auxiliary operators so raising the local concentration of the repressors and allowing the cell to synthesize only a small number of them (Müller-Hill, 1998); this assembly of repressors and DNA sequences is a quasi-equilibrium one (on the time scale of a bacterial generation) insofar as it continues to exist in the absence of a flow of energy. Such a hyperstructure would be relatively small, given that there are only ten or so LacI repressors in the cell, however, similar principles may give rise to much larger structures as evidenced by the foci formed by the abundant SeqA protein (Onogi *et al*., 1999) (see below).

Examples of other candidate hyperstructures include those associated with chemotaxis (Bray *et al*., 1998), where the size of the hyperstructure (they use other terms such as array, cluster and lattice) is implicated in the amplification of the signal, with glycolysis (Amar *et al*., 2002), where the formation of a hyperstructure to channel intermediates would both ensure efficient metabolism and prevent possible perturbations of other metabolic pathways, and with the synthesis of ribosomes, where rRNA genes appear to be grouped into hyperstructures in rapid growth conditions (Cabrera and Jin, 2003). In the case of the cell cycle, several lines of evidence point to the existence of a replication hyperstructure that would comprise the enzymes responsible for synthesizing the precursors of DNA, the enzymes responsible for DNA synthesis and even the genes encoding those enzymes (Guzman *et al*., 2002; Molina and Skarstad, 2004). One of the factors responsible for the formation of this hyperstructure could involve the SeqA protein, which forms large foci (Ohsumi *et al*., 2001), binding to hemi-methylated – that is, newly replicated – GATC sequences clustered near genes encoding replication or repair enzymes (Norris *et al*., 2000) and hence delivering the newly synthesized enzymes straight to where they are needed. Another hyperstructure concerned with replication is based on the Muk proteins which appear to be involved in the compaction of DNA (Ohsumi *et al*., 2001).

Yet another class of hyperstructures corresponds to the cytoskeletal-like networks. It is now evident that bacteria possess networks of the tubulin-like protein FtsZ (Thanedar and Margolin, 2004), actin-like proteins such as MreB and Mbl (Daniel and Errington, 2003), and even the elongation factor EF-Tu (itself a former 'actin' candidate) (Mayer, 2003). Intriguingly, the MinD protein itself, which is involved in cell division (see preceding section), also forms spiral filaments. The extent to which these can be considered as quasi-equilibrium or non-equilibrium structures is discussed below.

The question we must now ask is what is the advantage of thinking in terms of hyperstructures? How, in other words, does this intermediate level help us grapple with complexity? A general characteristic of an intermediate level is that it filters out noise and buffers information from the level below it and has properties that determine the

level above it (Lemke, 2000). Does this apply to hyperstructures? It has been argued for both prokaryotes (Guzman *et al*., 2002) and eukaryotes (Mathews, 1988) that a replication hyperstructure delivering precursors direct to the polymerases would protect replication from fluctuations in precursor availability. Indeed, a bacterium replicating its chromosome with two replication forks would consume around 3000 nucleotides per second with a very small pool of dNTP sufficient for replication for no longer than half a minute (Werner, 1971). Hence the higher level of the hyperstructure cushions the system from noise at the lower level of molecules. As regards the level of the hyperstructure determining events at the level above, it has been argued that initiation of replication could itself emerge from the dynamics of hyperstructures rather than from the dynamics of individual proteins (Norris *et al*., 2002a). In this proposal, the number of different hyperstructures decreases in the build-up to initiation because some lose out in the competition for resources and as these hyperstructures disappear they release a factor such as the initiator protein – and transcription factor – DnaA to trigger initiation. An important point here is that the protein (or other factor) serves as a messenger boy or postman in the communication between hyperstructures rather than as a commander.

To return to the question of how the existence of an intermediate level helps us grapple with complexity – or rather hypercomplexity – another advantage of an intermediate level of organisation is that it can integrate the simultaneous operation of many different organising processes; this is because these processes create the interacting structures characteristic of this level; indeed, these processes achieve their significance as *organising* processes insofar as they create, in this bacterial case, hyperstructures. Now consider the intermediate level of hyperstructures from the viewpoint of competitive coherence. One of the difficulties with the more sophisticated versions of competitive coherence is in generating the new state using several *Active* sets simultaneously. One solution is to change level, as mentioned above, such that each *Active* set (which corresponds to lower level macromolecules connected by a common factor) becomes a rather specialised hyperstructure dependent on only one organising process (such as a membrane domain of cardiolipin and particular proteins connected by binding to calcium) as opposed to a hyperstructure that results from several different processes. It is therefore important to build the concept of level into the parameters of competitive coherence if we are to use them to describe biological organisations. This is not of course new territory (even if we can give it a slightly different slant). Hierarchical complexity has a long history (Bonner, 1988).

In the next section, we shall examine the relationship between quasi-equilibrium and non-equilibrium hyperstructures and, in particular, how the dynamic balance between them determines the changing phenotypes of the cell.

## 6. BACTERIA ARE SELECTED FOR BOTH GROWTH AND SURVIVAL

A bacterial cell is a compromise solution between a robustness that maximises survival and an efficiency that maximises growth. Cells have to both endure long periods in hell and profit from brief periods in heaven. To survive in hell, they must adopt strategies that do not depend on the supply of energy whilst to flourish in heaven, they must be prepared to squander it (and to grow rapidly rather than efficiently). This compromise solution involves, in our view, quasi-equilibrium structures, which resist dissociation

in the absence of a flux of energy/nutrients, and non-equilibrium structures, which do require such a flux. The explanation is that (1) to survive difficult times cells contain quasi-equilibrium structures that allow the resumption of key functions for growth when times improve and (2) to grow rapidly and distance competitors, cells contain non-equilibrium structures that allow transport, transcription, translation, signalling etc. Quasi-equilibrium structures generate non-equilibrium ones as cells go from a survival to a growth regime and, *vice versa*, non-equilibrium structures generate equilibrium ones as cells go from a growth to survival regime. A nice example of this is the repair of double-stranded DNA breaks. It has been proposed that exposure to DNA-damaging agents results in the formation of a fibrillar RecA-DNA repairosome (alias a RecA hyperstructure) in which repair depends on energy-consuming processes such as exonuclease and unwinding activities; if exposure continues such that the rate of damage exceeds that of repair, ATP levels falls and the dynamic RecA non-equilibrium hyperstructure collapses into a RecA-DNA co-crystal or equilibrium hyperstructure in which the DNA is nevertheless protected (for references see (Minsky *et al*., 2002)).

The above DNA repair scenario illustrates how a non-equilibrium or a quasi-equilibrium hyperstructure is required in conditions where a useable source of energy is either abundant or scarce, respectively. More generally, a range of interacting non-equilibrium and quasi-equilibrium hyperstructures is needed to allow bacteria to confront a huge variety of environmental changes. There is an increasing amount of evidence that the chances of survival of bacteria are improved if the population possesses a phenotypic diversity such that there are always some bacteria ready to either exploit change or resist it (Balaban *et al*., 2004; Booth, 2002; Tolker-Nielsen *et al*., 1998). (Such ideas have a long history (Baldwin, 1896a,b).) There is a problem here: the combination of positive feedback and limited resources makes it likely that as the bacterium grows and advances towards DNA replication it becomes dominated by a small number of non-equilibrium hyperstructures which would make it vulnerable if conditions were to suddenly deteriorate (Norris *et al*., 2002a); we have speculated that bacteria avoid this vulnerability by sensing their metabolic status; such sensing could be achieved if the stability of an EF-Tu (Mayer, 2003) or FtsZ (Thanedar and Margolin, 2004) 'cytoskeletal' network were determined by the degree of activity of its constituents or associated enzymes. Changes in this network might then trigger the cell cycle which, at least in principle, is a powerful way of yielding daughter cells with different phenotypes equipping them for a wide variety of challenges, stresses and opportunities (Norris *et al*., 2002a; Segre *et al*., 2000). The argument here is that the existence of two chemically identical chromosomes in the same cytoplasm allows intracellular differentiation because there is competition between genes for access to RNA polymerase and between mRNAs for access to ribosomes (Norris and Madsen, 1995). Hence positive feedback circuits can operate whereby the expression of one of the two copies of a gene can increase its expression at the expense of the other copy. Factors responsible for linking the expression of genes that serve related functions (e.g. the functions related to growth in heaven) can lead to one coherent pattern of expression associated with the one daughter chromosome whilst another pattern of functions (e.g. related to survival in hell) is associated with the other daughter chromosome. It is then the task of chromosome segregation and cell division to put these differentially expressed chromosomes into separate cells. This argument is underpinned by evidence from the genome where genes needed for survival in stress conditions are carried on one strand whilst those needed for growth are carried on the

other; these findings underpin the strand-specific model in which genes on the same strand in the parental cell that are expressed together in a hyperstructure continue to be expressed together and segregate together in the daughter cell (Rocha *et al*., 2003). This would mean that each of the daughter chromosomes has a different set of hyperstructures associated with it and hence each daughter cell has, potentially, a different phenotype appropriate for growth or survival.

This model can be usefully married to one in which differences in the structure of the condensed daughter chromosomes are proposed to facilitate separation (Bouligand and Norris, 2001). In this marriage, the daughter chromosome with the "stationary phase" pattern of expression tends to a condensed, liquid crystal structure whilst the other daughter chromosome has the "exponential phase" pattern of expression leading to a dynamic structure that is immiscible with the condensed structure of the other. There is actually evidence for the simultaneous presence of daughter chromosomes with different structures in the radiation-resistant bacterium, *Deinococcus radiodurans* (Minsky *et al*., 2002).

The question here is how to represent quasi-equilibrium and non-equilibrium hyperstructures, such as the RecA-DNA co-crystal and the 'repairosome', respectively, in terms of the parameters of competitive coherence. In a sense, they are already implicit in the *Active* sets which can represent hyperstructures and which have connections to the environment. Hence, the quasi-equilibrium or non-equilibrium nature of the *Active* set is a parameter that can be detected and displayed.

## 7. APPLYING COMPETITIVE COHERENCE TO *E. coli*

Competition plays a large part in the phenotype of *E. coli* insofar as only a low percentage of genes are transcribed at any one time due to competition for the transcription apparatus (Shepherd *et al*., 2001; Stickle *et al*., 1994) whilst only some mRNAs are translated due to competition for ribosomes (Vind *et al*., 1993). In the simple – albeit false – vision of *E. coli* as an unstructured bag of genes and enzymes, the phenotype results essentially from the set of proteins synthesised and activated to perform their functions in the cell at any one time. From this point of view, the phenotype corresponds to a succession of sets of proteins as determined by transcription factors. Each of these sets of proteins creates a cell state. Competition in the form of two competing processes is, we contend, central to this succession of states (Norris, 1998). Let us consider first the *Next* process, namely, how one cell state generates the *next* state. The *Next* process reflects the successive nature of cellular events whereby, for example, a transcription factor is produced in one interval and the enzymes under its control are produced in the next interval. DNA damage, for example, activates the RecA protein (which cleaves the LexA repressor) to allow expression of the genes encoding the enzymes needed for DNA recombination and repair as part of the SOS response. Hence RecA activation in one state is followed by the presence of SOS enzymes in the next state. The *Next* process ensures continuity – genes are expressed in one cell state for their products to be used in the next. The possibility exists that, in a constant environment, feedback loops lead to some sets of genes being expressed continually (and others not at all) and in this case the *Next* process is maintaining the genetic *status quo*. The second process, the *Now* process, reflects the constraint on functioning enzymes to form an ensemble coherent with respect to both one another (internal coherence) and the environment (external coherence). In a

cell state in which the enzymes of the SOS response are repairing damaged DNA on one chromosome using undamaged sequences on the other, the *Now* process ensures internal coherence by preventing the chromosomes being segregated into separate daughter cells; hence, the *sfiA* gene is induced as part of the SOS response so that the protein it encodes can interact with FtsZ to inhibit cell division. As regards external coherence, a cell state is coherent if the cell has synthesized all the enzymes needed to transport and metabolise a particular substrate present in the environment (and not the enzymes needed for substrates that are not present!); hence *E. coli* growing in the presence of glucose needs the *pts* enzymes to transport and phosphorylate this sugar but does not need the enzymes for the transport and initial breakdown of lactose (which are not synthesised due to repression of the *lac* operon). The *Now* process ensures coherence where coherence applies to more than just a set of enzymes but to the entire contents of the cell and its relationship with its environment. By coherence, we mean that the total pattern of transcription, translation, enzyme activity, ionic distribution, lipid composition etc. at any one time makes sound environmental sense in terms of survival or growth.

In the competitive coherence model, these two processes, *Next* and *Now*, compete with one another to determine the cell state. (it may be helpful here to point out to those interested in evolution echoes of "coadapted gene clusters" (Mayr, 1954) and "evolvability" (Kirschner and Gerhart, 1998).) How might competitive coherence actually work in a bacterium? The general philosophy can be illustrated with reference to our speculative view of *E. coli* as a network of hyperstructures (we stress that our purpose here is to illustrate and the validity of these views is therefore largely irrelevant). There is a competition amongst potential hyperstructures struggling to come into existence or to remain in existence so that they can determine the cell state. Consider the ribosomal hyperstructures responsible for producing rRNA and tRNA which bring together the genes encoding this stable RNA (Cabrera and Jin, 2003; Woldringh and Nanninga, 1985) and which compete for existence with (putative) hyperstructures for the production of amino acids and other metabolites. The proportion of the cell's mass occupied by the transcriptional and translational machinery is dependent on expression of the growth-rate-dependent promoters (e.g. ones for the genes encoding rRNA and tRNA) and the activity of these promoters depends to a large extent on negative supercoiling. In fact, supercoiling affects the expression of many genes with relaxation of supercoiling increasing the expression of a hundred genes and decreasing that of two hundred others (Peter *et al*., 2004). Supercoiling is determined at the level of an individual promoter by many factors (see below). Hence the hyperstructures in the present state of the cell will help determine the global and local levels of supercoiling in the next state and these levels will, in turn, help determine which hyperstructures are maintained, created or disassembled. This *Next* connectivity in the form of supercoiling is not, however, sufficient. Supercoiling is also affected by external stimuli, such as osmotic stress, oxygen tension, nutritional shifts, and temperature change – giving rise to the speculation that supercoiling acts as a second messenger (Peter *et al*., 2004); hence, *uv* irradiation that damages DNA leads to a relaxation of supercoiling that in turn diminishes the ribosomal hyperstructures. Such relaxation also leads to the induction of the SOS system and the production of abundant proteins such as RecA to carry out DNA repair. Here, supercoiling is also acting as a *Now* process. Diminution of ribosomal hyperstructures and formation of an SOS hyperstructure would alter the availability of the wide variety of factors that determine the supercoiling at the growth-rate dependent promoters, factors

that include topoisomerases, nucleoid-associated proteins, transcriptional adaptors and monitors of supercoiling (for references see (Travers and Muskhelishvili, 2005)). In other words, the change in availability of these factors resulting from the change in the ribosomal hyperstructures affects the formation of other hyperstructures in the same cell state. This occurs in a coherent way because it is at the level of hyperstructures and not at the lower level of relatively independent genes and proteins.

To illustrate how competition between *Now* and *Next* processes might operate, consider the initiation of chromosome replication and the 'key initiator protein', DnaA, which is activated by cardiolipin. There are a great many DnaA boxes scattered throughout the chromosome although some of them are concentrated in the origin of replication. One popular model for initiation depends on a competition for DnaA protein between the boxes in the origin and those elsewhere (Hansen *et al*., 1991). DnaA has a role as a transcription factor activating, for example, *nrd*, *glpD*, *fliC* (and probably also *purR*, *araF*, *appY* and *mutH*) and repressing *dnaA* itself, *mioC*, *rpoH*, *uvrB*, *proS* and *guaB* (Messer and Weigel, 1997). Now suppose that in the build-up to initiation, as the mass to DNA ratio increases, the increasing density of RNA polymerases and ribosomes etc. results in an increase in the size of ribosomal hyperstructures at the expense of other hyperstructures (note this might involve a slight increase in supercoiling although this would be difficult to measure). The progressive demise of these other hyperstructures may have many diverse consequences including release of proteins such as DnaA and of lipids such as cardiolipin. There would then be a critical state in which the ribosomal and other hyperstructures created by the *Next* process are so dominant that enough elements are released from disappearing hyperstructures for an entirely new hyperstructure to begin to form via a *Now* process. Hence, the determination of the developing cell state by the *Next* process would give way progressively to its determination by the *Now* process as DnaA and cardiolipin are released, as DnaA is activated by cardiolipin and as DnaA binds to boxes in the origin to trigger the formation of a replication hyperstructure and the recruitment to it of the numerous enzymes needed for precursor synthesis, repair, recombination, unwinding etc. along with the genes that encode these enzymes. There is actually good evidence for a replication hyperstructure created to some extent by the polymeric SeqA protein binding to the clusters of hemi-methylated (i.e. newly replicated) sites in the above genes (Molina and Skarstad, 2004; Norris *et al*., 2000).

## 8. A BASIS IN PROGRAMMING FOR COMPETITIVE COHERENCE

One of the reasons for developing competitive coherence here in the context of an avant-garde approach to bacteria is to explore some of the characteristics of biological systems that could be built into an artificial learning system. More precisely, the idea is to see which concepts and findings from bacterial physiology might be implemented as parameters in a program; the possible contributions, if any, of these parameters at different values to the way in which the program 'learns' can then be evaluated in a variety of environments; finally, after evaluation *in silico*, the biological system can be re-examined. Some confidence that the concept of competitive coherence may be implemented with a modicum of success comes from previous work based on an artificial learning system in which local connections between neurons are strengthened as a result

of their membership of the equivalent of a limited *Active* set that contains a desirable output (Stassinopoulos and Bak, 1995).

## 9. MEASURING COMPLEXITY IN TERMS OF COMPETITIVE COHERENCE

Consider a cell moving through phenotype space where its phenotype is decided at the level of hyperstructures. There is a competition amongst hyperstructures to be included in the new cell state. This competition is based on the frequencies of two groups of connections, *Next* and *Now*, between hyperstructures. Each hyperstructure $i$ of the current cell state has connections $C$ to the other hyperstructures, $j$, in the cell. The *Next* group of connections is obtained from the connections between a hyperstructure present in one cell state and those hyperstructures present in the following cell state. By summing the *Next* connections for each member ($\Sigma^{NEXT}C_{i,j}$), the entire set of possible hyperstructures might, in principle, be ranked in order of their degree of connectivity to the set of hyperstructures actually present in the current cell state. The hyperstructures with the highest connectivities can then be selected to participate in the new cell state. This ensures a strong relationship between hyperstructures in successive cell states and hence a continuity. Moreover, members of the present state are likely to be reselected by this process hence its *status quo* nature.

Once a few hyperstructures have been selected to participate in the future cell state, the *Now* process starts to play a role. The *Now* group of connections is obtained from the connections between hyperstructures that are present together in the same cell state ($\Sigma^{NOW}C_{i,j}$). Each member of the new, as yet incomplete, cell state is examined for connections to other candidate hyperstructures with which it is regularly present in a state and, by summing the connections for all these new hyperstructures, the members of the entire set of hyperstructures can again be ranked, this time in order of their degree of *Now* connectivity. This gives an internal coherence to the set of hyperstructures actually chosen to be present together.

Initially, the *Next* process dominates selection for the cell state with those present in the current state 'deciding' on some of those that are to participate in the next state. However, there may be an important input from the environment resulting, for example, from exposure to *uv* radiation. Once a few hyperstructures have been chosen via the *Next* process and environmental stimuli, the relationship between hyperstructures that can cooperate, the *Now* process, plays an increasing role and the composition of the cell state becomes a competition on the basis of connectivities between the two processes. This means that the composition is selected so as to be consistent over successive states and to be coherent with respect to the particular state.

In this examination of competitive coherence at the hyperstructure level, each hyperstructure can therefore be given an integer value corresponding to the number of connections it has to other hyperstructures that exist at the same time or that exist in the following cell state. In the framework of this vision, we can now suggest candidate parameters for hypercomplexity that are based on competitive coherence. These would include the total number of different hyperstructures in the bacterium, the number of different hyperstructures that are present in the average cell state and a measure of the types of connectivity operating in the network of hyperstructures.

Many, if not most, types of biological organisation are selected to both grow in favourable conditions and to survive harsh ones. We have argued above that this is also the case for bacteria which contain both non-equilibrium and quasi-equilibrium hyperstructures. We have also argued that the proportion of cell mass in the form of non-equilibrium or quasi-equilibrium hyperstructures varies during the cell cycle and that chromosome replication and cell division help maintain the ratio of these two classes (Norris *et al*., 2002a). In other words, the ratio of non-equilibrium to quasi-equilibrium hyperstructures is an important parameter in bacterial physiology and an expression for bacterial hypercomplexity should also take the non-equilibrium and quasi-equilibrium nature of hyperstructures into account.

This is not the end of the story. At the level of an individual hyperstructure, the proportions of non-equilibrium constituents or quasi-equilibrium are also variable. Within a putative glycolytic hyperstructure, there may be enzymes that only associate with one another in the presence of substrate as well as enzymes that associate to form stable dimers (corresponding to successive enzymes in the pathway) irrespective of substrate. Finally, of course, there are the different levels of organisation to be considered if we are to take into account hierarchical complexity. This leads us to propose a set of general parameters at a given level $i$ in terms of the constituents of the level:

$T_i$ the total number of constituents

$N_i$ the number of non-equilibrium constituents actually present in the *Active* set

$Q_i$ the number of quasi-equilibrium constituents actually present in the *Active* set

$C_i$ is a measure of the types of *C*onnectivity operating in the network of the constituents

## 10. EMERGENCE

Emergent properties are properties that cannot be reduced to those of the constituents of the system and they resist attempts to predict or deduce them (Van Regenmortel, 2004). Emergence acquires its explanatory force when it is accepted that higher level properties possess a causal efficacy of their own and causality is not restricted to their dependence on lower level phenomena. In the framework of competitive coherence, emergence is related to the formation of the new state, the subset of elements that are active together. Suppose each constituent has a large number of characteristics. This is clearly the case of macromolecules such as mRNA and proteins which contain a large number of sites that can bind water, ions, molecules and other macromolecules. As proteins are being chosen via competitive coherence to work together, suppose that the first ones to be chosen just happen to contain a binding site to the same molecule. Suppose that, in some environments, this combination of proteins proves useful. Suppose too that this molecule becomes available, perhaps for the first time. The presence of this binding site could then become an important factor in the coherence process which dominates the choice of the rest of the proteins to work together in the *Active* set. In other words, the environment acts via the coherence process to lend importance to one out of many sites. The result is the selection of this site (plus the molecule that binds to it) as a determinant of the cell's response to a particular environment. More specifically, consider, for example, that (1) this binding site is for a particular phospholipid with long, saturated acyl chains and (2) the proteins with this site bind to the phospholipid to form a domain in which they are juxtaposed and in which their

activities complement one another. There might then be a selection for this binding site in other complementary proteins. In the language of competitive coherence, binding to this phospholipid would become a type of connectivity to determine membership of an *Active* set and this *Active* set would take on the physical form of a proteolipid domain responsible for a particular function. Hence emergence in the context of competitive coherence can be understood in terms of a new criterion for membership of the *Active* set.

## 11. DISCUSSION

What vocabulary and what parameters do we need to begin to classify complex living systems? *E. coli* is a paradigm of a complex living system. Non-linearity, random fluctuations due to small numbers of key elements, historical accidents, emergence and other concepts used to characterise complex systems are all relevant to its behaviour. However, analyses of its behaviour are generally limited to a particular aspect which is often considered a complex system in its own right and which is often explained in terms of the operation of a small number of variables. This misses, we believe, the essence of biological complexity which can only be explained satisfactorily in terms of many variables. Cell division, for example, depends on a multitude of processes that may include lipid domain formation, the polymerisation and depolymerisation of several proteins, ion fluxes and ion condensation onto polymers, transcription and translation, and possibly gel/sol transitions. In struggling to understand and evaluate the complexity of a bacterium, we therefore need to take into account that the organisation at a particular level – for example that required for cell division – is the result of many processes. It is *hypercomplex* rather than complex. We therefore need a term to reflect the operation of many processes. We also need to take into account the existence of different levels of organisation within biological systems such as bacteria. One of these levels is, we speculate, that of hyperstructures which are extended multi-molecule assemblies responsible for performing functions such as the transport of a nutrient or the act of cell division. The parameters of hypercomplexity must therefore contain a term for levels. There is still more to take into account. *E. coli*, like many human organisations, is selected to grow in good times and to survive through hard ones. These conflicting constraints are met in part by the bacterium having both stable, quasi-equilibrium structures that need no flux of metabolites and unstable, non-equilibrium structures that do need such a flux. In the bacterial scenario, each of these sets of structures can generate the other.

What parameters should be chosen for hypercomplexity to take into account the aspects of organisations mentioned above? We suggest that the concept of competitive coherence may be helpful. Many biological organisations, from bacteria to research laboratories and football teams depend on the choice of active groups of expressed genes, working scientists and performing players, respectively, out of a larger pool of candidates via a competition between two processes. The first of these processes is one which ensures continuity between the successive groups so that they do not fluctuate wildly in composition. This gives them a meaningful history in which the *status quo* is important. The second process ensures that the group that is selected is a coherent one in respect of both its own composition and the relationship of this composition to the environment. Given the evolution of organisations between growth

and survival, a hypercomplex analysis should also contain parameters that represent the quasi-equilibrium or non-equilibrium nature of constituents of the active group. The parameters involved in competitive coherence are $\mathbf{T}_i$ the total numbers of constituents, $\mathbf{N}_i$ and $\mathbf{Q}_i$ the number of non-equilibrium and quasi-equilibrium constituents, respectively, in the active group or team, and $C_i$ the number of types of processes or connectivities operating to choose the members of the active group. (We do not consider further here the relationship between complexity and connectivity – see for example (Raine *et al.*, 2003)). In a hypercomplex analysis, these parameters might, in principle, be determined at each level $i$ within the organisation. Although our approach is not valid for all systems termed complex, it may be useful for those in which an active set is chosen from a large number of potential constituents. By restricting our description of complexity to one level so that the constituents are considered as modules (and so ignoring their internal complexity), we can use the same parameters of $T$, $N$, $Q$ and $C$ to quantify the complexity of very different complex systems. We might, in principle, compare the complexity of the functioning neuron with that of the functioning brain – or even compare the complexity of a growing bacterium to that of a government.

The idea of the evolution of complex systems at the edge of chaos has been influential. One of the powerful conclusions of this is that two important parameters in determining the behaviour of a model genetic network are the number of genes and the number of links between them. When the number of links is two, the model falls into neither a frozen state in which no genes change state nor a continually changing state in which no patterns of gene expression are repeated. Biological organisations, however, are hypercomplex and do more than evolve subject to the constraints of a network in which all genes can be active simultaneously. An alternative or complementary vision is one in which only a subset of the network can be active at any one time. Such organisations are typically subject to selection in an environment that is rarely constant. This means that there are at least two other axes which describe the evolution of many hypercomplex systems. The first is the survival/growth axis (alias robustness/efficiency or non-equilibrium/quasi-equilibrium). In this case, bacteria have adopted strategies that include varying the non-equilibrium/quasi-equilibrium nature of their hyperstructures and redistributing these structures during the cell cycle to give daughters with different phenotypes (at the level of populations there is also the story of spontaneous mutators). The second is the consistency/coherence axis (alias history/present). In this case, it is easy to see how bacteria can satisfy the constraint of consistency by having the genes responsible for one phenotype being directly coupled (for example, via transcription factors) to the genes responsible for the next phenotype. Satisfying the coherence constraint to coordinate the expression of genes with both one another and with the environment might again be achieved by prosaic means although there are more exotic possibilities such as those offered by ion condensation onto charged linear polymers (Ripoll *et al.*, 2004) or by collective oscillations in which the cell becomes a giant dipole (Norris and Hyland, 1997).

Emergence may also find its place in the framework of competitive coherence developed here. As a new combination of constituents is being selected to form the active group, a new factor such as a binding site that is common to many of the constituents, may start to assure coherence with the environment. This opens up the possibility of beginning to model emergence using the powerful systems of artificial chemistry (Segre *et al.*, 2000) and artificial microbiology (Demarty *et al.*, 2002).

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