Questions for cell cyclists

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There are respectable questions that are asked about the bacterial cell cycle, though not as frequently as one might like. What is the nature of the signal that initiates chromosome replication? What ends the period of sequestration of newly replicated origins of replication? What couples chromosome replication/segregation to cell division? What initiates cell division? What times the formation of the FtsZ ring and positions it at the equator or elsewhere? Is there an inactivation mechanism that prevents multiple rounds of cell division? Biochemists and molecular biologists often try to answer these questions in terms of biochemistry and molecular biology. Such attempts will fail if the concepts needed are to be found in other disciplines. There are also less respectable questions that are not generally asked. What is the cell cycle *for*? Could another function emerge from the cell cycle beside that of simply increasing the number of genetically identical cells? If cells initiate chromosome replication at a particular mass (origin/mass etc.), why one particular mass rather than another? Are 'key' regulator proteins simply the messenger boys instructed by the dynamics of structures? Are these proteins just part of a molecular overlay that behaves as a coupled oscillator? What *is* a cell? Satisfactory answers to the problems of the bacterial cell cycle might be expected to be answers to other problems in cell biology and to elucidate for example the eukaryotic cell cycle, nutritional shiftup, or the origin of life. Deeply satisfying answers might be founded in the nature of cells themselves. Here we conduct a magical mystery tour of a few speculative ideas.

Keywords: bacteria, cell division, chromosome segregation, DNA replication

1. INTRODUCTION

The nature of the regulation of the cell cycle has been one of biology's more profound mysteries. For several years, it seemed that the bacterial cell cycle had been largely understood. Initiation of chromosome replication resulted from either the dilution of a repressor or the accumulation of an activator. Chromosomes were segregated by the growth of the peptidoglycan wall between the origins of replication that attached them to this envelope. Cell division resulted from invagination of the wall. The discovery that cell could happily replicate scores of minichromosomes should have sunk the simple models for initiation of replication. Worse, the signal responsible for the initiation of the replication of minichromosomes continues 'as normal' even when the replication of the chromosome itself is random [1]. Despite this, these early models have not been replaced. The mysteries underlying chromosome segregation and cell division have fared better due to the discoveries of proteins with similar activities and/or structures to their eukaryotic equivalents; indeed a new paradigm for division site selection is now based on the Min proteins, which when defective lead to misplaced division and minicell production and which can oscillate from pole to pole [2]. The readiness with which this Min hypothesis has been embraced may herald a serious interest in the value of theory and in the possible relevance of concepts found in physics and physical chemistry, although it may of course simply reflect a lack of critical interest. Here, we give examples of concepts and approaches that may be relevant by trying to answer a few simple questions.

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2. WHAT IS A BACTERIAL CELL?

It has been argued that the cell cannot be understood adequately as anything other than itself [3]. In other words, the cell cannot be reduced to a single simplified model system without losing its essence. Although we have to simplify our visions of the cell in order to study it, it must be stressed that the cell is far more than any of the definitions below. The cell is neither a neural net nor an oscillating system of diffusible enzymes nor a dissipative system nor a set of phase-separated membranes and cytoplasm etc. Rather, the cell is the creator and the creation of an extraordinarily high density of different organizing processes that have autocatalytic relationships with one another. It is a system that produces self-organization and assembly by recruiting and dismissing a multitude of processes and molecules. Whatever does this is a cell and, for the moment, we only know one example: the biological cell. In other words, the cell is its own metaphor [3].

2.1 An autocatalytic network?

All cells that grow have to be autocatalytic networks in which the elements catalyse their own synthesis from a starting foodset [4]. In fact, the bacterial cell may contain several autocatalytic networks—could they interfere with one another? One network might, for example, consume a key element in another and, if so, how are they reconciled? Is there a biologically realistic way in which different intracellular networks might undergo a phase separation based, for example, on connectivities (see § 2.4)?

2.2 A device to explore phenotype space?

Put differently, a cell is a compromise solution between a robustness that leads to maximal survival and an efficiency that leads to maximal 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, equilibrium structures, which resist dissociation in the absence of a flux of energy/nutrients, and non-equilibrium structures, which do require such a flux. One possibility is then that (1) to survive difficult times cells contain equilibrium structures that allow the resumption of key functions for growth when times improve [5], and (2) to grow rapidly and outdistance competitors, cells contain non-equilibrium structures [6].

Equilibrium structures generate non-equilibrium ones as cells go from a survival to a growth régime and, *vice versa*, non-equilibrium structures generate equilibrium ones as cells go from a growth to a survival régime. In this approach, the cell can be described as a ratio of equilibrium and non-equilibrium structures (or hyper-structures, see below) and the question of exploring phenotype space becomes "How are equilibrium and non-equilibrium functions balanced?"

2.3 A molecular form of competitive coherence?

The functioning of many organisations is characterized by a succession of states; the elements of each state are generated by a competition for inclusion in the new state between two sets of influences dependent on (1) the members of the old state, and (2) the progressive coherence of those selected [7]. Consider the problem of selecting an amateur football team each week from a larger group of potential players. One influence in deciding who plays next week is who plays this week (e.g. due to shared transport arrangements) and the other influence is the coherence of the team (must have a goalkeeper, midfield player and striker).

2.4 A particular pattern of connectivity.

A cell—indeed any object—has a frontier with the exterior that can be defined by the drop in average connectivity at this frontier; that is, the bits inside the cell are more connected with one another than they are with the bits outside the cell. (We concede that in some contexts the cell is highly connected to its microenvironment and cannot be meaningfully dissociated from it.) What is the dynamic pattern of connectivity that describes cellular processes and the relationships between them [8, 9]? Is it related, for example, to the small world of metabolism and to transcription characterized by a power law with a particular slope [10]? And do cells that are replicating their chromosome (or that have already replicated it) have *two* distinct patterns of connectivity corresponding to the "exponential and stationary phase" destinies of the future daughter cells?

2.5 A tensegrity structure?

In this architectural approach, the integrity of the eukaryotic cell is maintained by elastic filaments that ensure continuous tension throughout and rigid struts to resist local compression [11]. In an approach to the origins of life in which a capacity to resist turgor and mechanical stresses is given importance, cells originated as tensegrity structures and transcription and translation

originated as processes that maintain cellular integrity [12]. In our approach to modern bacteria in which "exponential and stationary phase" daughter cells are generated by the cell cycle, we might expect correspondingly different patterns of tensegrity—but what are these patterns?

2.6 A unit of subjective experience?

What exactly is 'subjective experience' and how can it be related to life and indeed to the material world? How far down the scale does subjective experience go [13, 14]? And could it play any rôle in intracellular organisation? What experiments could be done to decide whether such questions are stupid?

3. WHAT ARE THE FUNCTIONS OF THE CELL CYCLE?

The answer to this question depends on what a cell is. Here, we consider just two of the above definitions, namely that a cell is one or more autocatalytic networks (§ 2.1) and a device to explore phenotype space (§ 2.2). Following studies of artificial autocatalytic networks, it has been proposed that cell division originated in its capacity to separate different autocatalytic networks that may interfere with one another [15, 16]. There may be more to it than this. In our hypothesis, firstly, the cell cycle has a major rôle in the generation of the phenotypic diversity that allows a bacterial *population* to be ready for a wide variety of challenges, stresses and opportunities because the members of this population are born with the appropriate phenotypes. Secondly, the cell cycle responds to an excessive development of non-equilibrium activities/structures and restores a robust ratio of equilibrium to non-equilibrium structures and functions; in other words, the cell cycle adjusts the economy of the cell and prevents it getting "overheated" and falling victim to minor fluctuations in the environment. There is some evidence for the natural heterogeneity of bacterial populations even in conditions of unnatural homogeneity [17, 18] (for other references see [19]). One way to generate this heterogeneity is via stochastic variation in the small numbers of certain regulatory elements [20]. Another way is via the successive events of the cell cycle [6, 15] and, in our hypothesis, the existence of two chemically identical chromosomes in the same cytoplasm allows intracellular differentiation [21]. This is essentially because there is competition between genes for access to RNA polymerase and between mRNAs for access to ribosomes. 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 (consider the analogy of two neighbouring laboratories competing for limited funding ...). 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 [21]. It is then the task of chromosome segregation and cell division to put these differentially expressed chromosomes into separate cells.

4. WHAT IS THE NATURE OF THE SIGNAL THAT INITIATES CHROMOSOME REPLICATION?

One of the key criteria to use in evaluating candidates for the signal initiating chromosome replication is whether it is easy to see how it arose during evolution. For example, our evaluation of a potential signal would be heavily weighted in its favour if it were to have the physico-chemical characteristic of pushing the dNTP monomer to DNA polymer reaction to the right, because then one could see how this particular signal might have been selected originally. The candidates given below should be similarly evaluated.

4.1 Transcriptional sensing?

If the function of DNA is to be transcribed, then we might expect a relationship between replication and transcription. For example, it might be supposed that the cell senses when DNA risks becomes limiting and therefore initiates chromosome replication. This is of course a vision of the cell in which selection is at the level of performance in Olympic games in heaven rather than in the grimmer game of survival in hell (see Robustness sensing, § 4.3, below). It turns out that this simple version of transcriptional sensing is—along with a whole class of other models—wrong, as shown by a key experiment in which the synchronous initiation of replication of scores of minichromosomes is unperturbed in cells in which initiation of replication of the chromosome itself is seriously perturbed [1].

4.2 Diversity sensing?

An alternative, based on definitions § 2.1 and § 2.2 is that the growing cell senses when its diversity has diminished and initiates chromosome replication to restore this diversity [6] (consider the analogy of what can happen in a conservative family business when an additional boss arrives). How could such sensing of diversity (alias complexity?) occur? One way would be

to have a transcription factor that could also serve as an initiation factor, for which the DnaA protein in *E. coli* would be a candidate. Liberation of the transcription factor by a reduction of the number of functions in which it is involved (i.e. reduction of diversity) could free the factor to trigger replication.

4.3 Robustness sensing?

A related idea is that the cell growing in heaven invests increasingly in non-equilibrium functions and structures at the expense of equilibrium ones. Replication then restores the equilibrium structures required for survival. Again, sensing the change in intracellular conditions could be done by the availability of a transcription/initiation factor (see above). Interesting alternatives include the ratio of the constituents of RNA and DNA (or precursors of these constituents) based on the idea that fast growth is accompanied by (1) a characteristic investment in rRNA and certain species of mRNA and (2) an increasing consumption of GTP for protein synthesis. There is of course the possibility that the signal is of an entirely different nature involving, for example, a change in water structure brought about by increasing ATP hydrolysis.

5. WHAT IS THE NATURE OF THE SIGNAL THAT CONTROLS CELL DIVISION?

Just as with the initiation of chromosome replication, candidates for the signal should be evaluated according to the criterion of whether it is easy to see how the signal might have evolved. In virtually all cells, division is an affair of membrane dynamics and often seems to involve tubulin or tubulin-like proteins such as the bacterial version, FtsZ. FtsZ is the key protein in bacterial cell division and attempts to find a protein acting upstream of FtsZ have failed.

5.1 Proteolipid domains?

The bacterial membrane has both a structure and a structure that changes during the cell cycle. These changes are related to the presence [22] and the activity [23] of the chromosome. A physico-chemical basis for the polymerization of FtsZ at the membrane in cell division might therefore reside in proteolipid domains created by the coupled transcription-translation-insertion or *transertion* of proteins into and through membrane [22–26]. For example, those phospholipids not bound to nascent proteins being inserted into the membrane around the chromosomes might congregate in the centre of the cell and create a membrane domain to which FtsZ would bind preferentially (Figure 1).
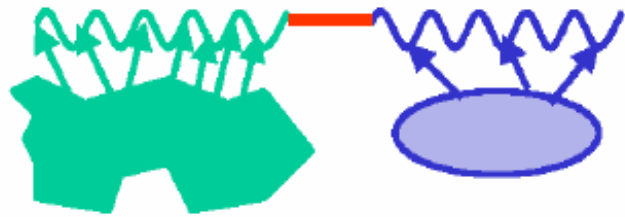

Figure 1. Transertion (arrows) from chromosomes with different activities and structures creates membrane domains around them (wavy lines) and creates a division domain of specific phospholipid composition and structure between them (thick line).

5.2 Metabolic sensing?

As argued above for robustness sensing, it might be advantageous for a cell to sense its metabolic state. Consider, for example, a cell that has its resources shared out between two sets of functions related to fast growth and to survival: such a cell could use division to concentrate its resources in a daughter equipped for fast growth. There would be no point doing this if its metabolism were relatively untaxed. One way metabolic sensing could occur would be if there were an enzoskeleton involving FtsZ such that FtsZ polymers in the cytoplasm associated with enzymes of the general metabolism would, at a critical level of metabolic activity, break down to release FtsZ monomers to initiate cell division [25]. This would constitute a *structural sensor*. It is therefore significant that there is a fine FtsZ skeleton in moss chloroplasts [27] and an association between the tubulin cytoskeleton and glycolytic enzymes in eukaryotes [28].

6. ARE WE ASKING THE QUESTIONS AT THE RIGHT LEVEL OF INTRACELLULAR ORGANISATION?

A hyperstructure is a collection of diverse molecules (genes, mRNAs, proteins, ions, lipids) that is associated with at least one function (Figure 2). Certain hyperstructures may therefore structure cytoplasm, chromosome and membrane. In particular, 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 [29]. We have proposed previously that in the case of the phosphotransferase system in *E. coli* a non-equilibrium hyperstructure may form due to an increase in the affinity of its constituent enzymes for one another in the presence of the sugar. Briefly, enzymes E1 can only diffuse in the plane of the membrane whilst the other enzymes, E2 to E7, diffuse in the cytoplasm. The binding of the sugar substrate to the E1 enzymes leads to an increase in their affinity for one another and their
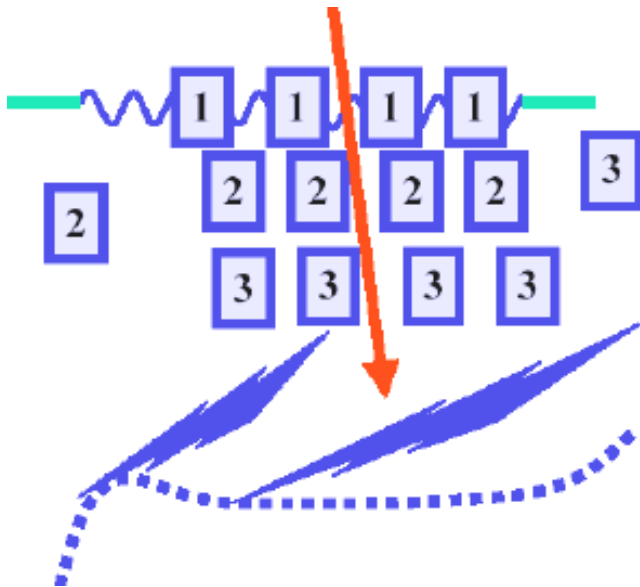
Figure 2. Formation of a hyperstructure involving genes, enzymes and the membrane, which is dependent on the presence of substrate. Enzymes (rectangles 1, 2 and 3) in the pathway assemble in the presence of substrate to transport and metabolize it (arrow). Enzyme 1, a membrane protein, structures the membrane (wavy line). The genes encoding these enzymes are attached to the hyperstructure by coupled transcription-translation (jagged shapes).
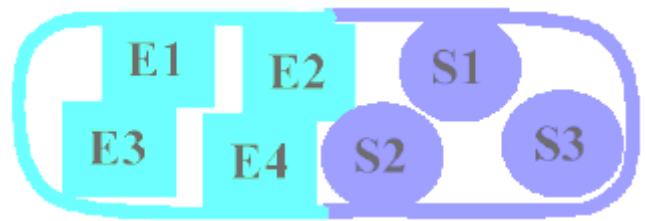

Figure 3. Segregation of hyperstructures. Rectangles lettered E correspond to hyperstructures that have common preferences for ions, lipids, binding proteins, water structures etc. and that ensure the "exponential phase" set of phenotypes whilst circles lettered S correspond to hyperstructures that ensure the "stationary phase" phenotypes and that have a different set of preferences.
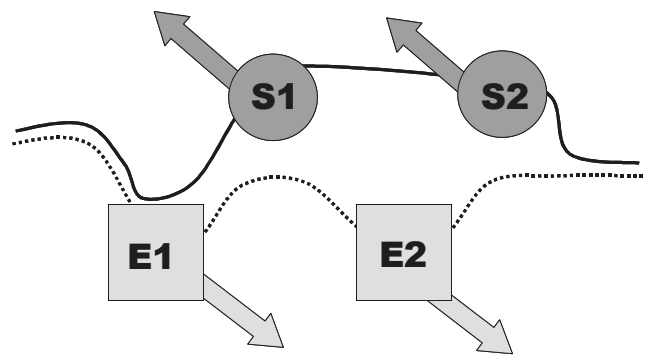

Figure 4. Strand-specific segregation mechanism. The hyperstructures S for the "stationary phase" set of functions are encoded by genes located on one strand of DNA (continuous line) whilst those for the "exponential set" E are on the other strand (dotted line).

assembly into an E1 domain. On binding its substrate, each enzyme in the pathway acquires an increased affinity for the following enzyme. This results in the assembly of the hyperstructure, which may be helped by the transcription of the genes encoding E1 to E7 and the simultaneous translation of the resultant mRNA. In the hyperstructure approach, cell cycle progress is considered to be a state cycle of hyperstructures and the generation of phenotypically different daughter cells results in part from interactions between hyperstructures (Figure 3). Such interactions can be invoked to explain how another cell cycle event, chromosome segregation, allows hyperstructures to create phenotypic diversity. For example, a *strand-specific* model can be based on each of the future daughter chromosomes being associated with a different set of hyperstructures in an asymmetric cell [30]. The essence of this segregation mechanism is that the 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 (Figure 4). The model requires an asymmetric distribution of *classes* of genes and of binding sites and other structures on the strands of the parental chromosome. There is some evidence for this distribution insofar as highly expressed genes encoding functions needed for rapid growth are preferentially located on one strand whilst those needed for resistance

to stresses are located on the other. This model can be usefully married to one in which different cholesteric pitches of the condensed daughter chromosomes have been proposed to facilitate separation [31]. 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 an "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* [5].

7. DISCUSSION

For far too long in studies of the bacterial cell cycle absence of evidence has been construed as evidence of absence. The absence of techniques (or the absence of *use* of techniques) that might have revealed heterogeneity at the level of structures and functions has been construed as evidence for the absence of heterogeneity.

This has led to hypotheses tinged with molecular vitalism and dominated by a vision of the bacterium as a cell with an unstructured cytoplasm surrounded by an unstructured membrane in which intelligent proteins diffuse freely. This vision has fitted in well with a number of other reductionist visions that include the RNA world in the case of the origin of life and the dance of sigma factors and other magic molecules in the case of differentiation. The last couple of decades have seen a gradual retreat from the idea of homogeneity under the pressure of increasing evidence for structured cytoplasm and membranes. This retreat has, however, yet to be translated into a readiness to generate or consider seriously hypotheses for cell cycle regulation that are based on dynamic structures (as opposed to those based on the simple diffusion of molecules). Here, we have advocated approaches to developing new hypotheses that are based on physics and physical chemistry. We have explored candidate hypotheses in the highly speculative context where we suggest that one partial answer to the question "what is a cell?" is "two autocatalytic networks with different connectivities that, in the form of equilibrium and non-equilibrium hyperstructures, explore phenotype space so as to both survive in hell and multiply in heaven". We argue that hypotheses for cell cycle regulation should be evaluated by the insights they provide into other fundamental problems. On this note, we suggest that hyperstructures are not too dissimilar to the non-covalent assemblies of monomeric mutually catalytic molecules, termed composomes [15], and other structures proposed to be the selectable units in the origin of life [32].

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