Hyperstructures 2008-2009

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1 Introduction

Many functions in both prokaryotes and eukaryotes are performed by large structures in which molecules, macromolecules and ions are physically associated [23]. In the case of *Escherichia coli, Bacillus subtilis, Caulobacter crescentus* and other model bacteria, examples of such hyperstructures include: the array of chemotaxis-specific receptors (Tar, Tsr, Trg, Tap, and Aer) [4]; dynamic, coupled transcription-translation and transcription-translation-insertion (transertion) hyperstructures comprising active RNA polymerases and ribosomes along with the nascent mRNAs and nascent proteins and indeed the highly expressed genes themselves [3]; the cytoskeletal filaments MreB, CreS and FtsZ; filaments of elongation factor EF-Tu [20]; metabolons of glycolytic enzymes [33]; foci of ATP synthase and succinate dehydrogenase in the membrane [15]; foci of the enzymes E1 of the phosphoenolpyruvate:sugar phosphotransferase system; clusters of secretion enzymes such as SecA; nucleofilaments of recombination enzymes such as RecA; the cell division machinery (comprising a lipid domain and proteins such FtsZ, FtsA, FtsI, FtsK and AmiC); the DNA replication factory (comprising enzymes such as PolC, DnaB, DnaG and DnaE as well as enzymes responsible for the synthesis of precursors such as ribonucleoside diphosphate reductase); cellulosomes and polycellulosomes [10]. Ambiquitous enzymes can occupy two different positions in the cell [21] and some hyperstructures depend on such enzymes and are functioning-dependent structures that assemble only when functioning and that disassemble when no longer functioning [31]. Other hyperstructures are equilibrium or quasi-equilibrium structures that remain even in the absence of a flow of energy or nutrients.

It has been proposed that hyperstructures constitute a level of organisation intermediate between macromolecules and the bacterial cell itself [23]. Communication between hyperstructures would then take the form of changes in: DNA supercoiling (which may be modulated by the transcription in one hyperstructure to affect the transcription needed for another hyperstructure), ion

condensation on charged filaments (which by being concentrated in stabilising one hyperstructure may be in short supply for another hyperstructure), signalling molecules (which may be emitted by hyperstructures), water structures (which may lead to the formation of one hyperstructure at the expense of another), and distribution of membrane domains (whereby a hyperstructure with particular lipid preferences may stabilise another with the same preferences). At this intermediate level of organization, hyperstructures would control the phenotype and, in particular, the bifurcations that occur, as during the cell cycle, so that events take place in the right place, at the right time and in the right order.

Exploration of the hyperstructure concept may also prove useful for understanding eukaryotic cells. Interactions between hyperstructures in eukaryotes have been invoked to explain the structure and functioning of mitochondria and to explain the existence of mitochondrial DNA [32]. Members of the hyperstructures group work on podosomes, which are important in motility and mechanotransduction [9] and on the nucleolus, which has a multitude of roles in addition to the assembly of ribosomes [29]. Here we review briefly new discoveries about hyperstructures and describe some of the work being done by members of the hyperstructures group.

2 New hyperstructures

Evidence for new hyperstructures or for new aspects of known hyperstructures since our reviews in 2007 includes:

1. RNaseE is the main component of the RNA degradosome of *Escherichia coli*, which plays an essential role in RNA processing and decay. The degradosome also contains RNA helicase B, polynucleotide phosphorylase and, intriguingly in view of possible interactions between hyperstructures, the glycolytic enzyme, enolase. It has now been found that the degradosome forms helical filaments just under the membrane that may regulate access to substrates to prevent uncontrolled degradation [30]. The significance of the two hybrid finding for interaction between RNaseE and MinD (part of the Min hyperstructure for regulating cell division) remains to be determined.
2. Carboxysomes in bacteria such as *Synechococcus, Synechocystis* and *Halothiobacillus* enhance autotrophic carbon fixation via the Calvin cycle and are widely distributed among chemoautotrophs and cyanobacteria [7]. These hyperstructures, which the authors term 'microcompartments', are about 80-150 nm in cross section and are bounded by a 3-4 nm thick protein shell. Their mass is about 300 MDa and they are composed of several thousand polypeptides of 10-15 different types. There

is no evidence they contain lipids, RNA or DNA. The interior of the carboxysome contains the sequential metabolic enzymes carbonic anhydrase and ribulose bis-phosphate carboxylase monooxygenase. Carbonic anhydrase converts HCO3 to CO2 within the carboxysome, then ribulose bis-phosphate carboxylase monooxygenase converts CO2 and ribulose bisphosphate to 3-phosphoglycerate. The shell of the carboxysome may prevent diffusion of CO2 (and exclude O2) and so keep it close to the ribulose bis-phosphate carboxylase monooxygenase.

3. Pdu microcompartments or hyperstructures are responsible in bacteria such as *Salmonella enterica* for B12-dependent 1,2-propanediol utilization (pdu) [7]. They are 100-150 nm in cross section with a 3-4 nm protein shell. Their mass is about 600 MDa and they are composed of about 18,000 individual polypeptides of about 14-18 different types. They are believed to sequester an intermediate of 1,2-propanediol degradation (propionaldehyde) to prevent toxicity and diffusive loss. 1,2-propanediol degradation is also implicated in the pathogenesis of *Salmonella* and *Listeria*. In *S. enterica*, the genes involved in 1,2-propanediol degradation form a contiguous, twenty-three-gene cluster, one of the largest clusters of functionally related genes in this organism. It might be expected that coupled transcription, translation and assembly would mean that genes, nascent mRNA and nascent protein would at some stage be physically coupled to the Pdu hyperstructure yet, like carboxysomes, the Pdu hyperstructures are not believed to contain RNA, DNA or lipids. At least, not at present.

4. Eut microcompartments or hyperstructures are responsible in S. enterica for B12-dependent ethanolamine utilization (Eut) [7]. Ethanolamine utilization begins with conversion of ethanolamine to acetaldehyde by the B12-dependent enzyme, ethanolamine ammonia lyase, after which acetaldehyde is degraded to acetate and ethanol by a pathway analogous to 1,2-propanediol degradation. Overall, the degradation of 1,2-propanediol and ethanolamine share many features with the main difference being that 1,2-propanediol is a C3 compound and ethanolamine is a C2 compound. It has been suggested that the Eut hyperstructure concentrates substrates and enzymes to either increase metabolic efficiency or regulate metabolite levels.

5. Pyr hyperstructures are involved in *E. coli* in the phosphorylation of uridine monophosphate [35]. PyrH and MetK formed discrete protein foci within the cell as well of which 1 or 2 moved around the cytoplasm in a circles. PyrH also regulates the transcription of carbamoyl phosphatase and is thought to play a key role in chromosome partitioning and cell division.

6. The 'stressosome' in *B. subtilis*, a 1.8-megadalton hyperstructure of many different proteins that integrates a variety of signals to effect a single outcome [19]. These include the Rsb proteins which interact physically with one of the enzymes responsible for DNA replication, DnaE (Laurent Janniere, unpublished)
7. Podosomes are transient intracellular organelles with a biological role that is the subject of much active research and debate. Podosomes display many features of hyperstructures, notably as potential functioning-dependent structures. Indeed, podosomes are actin-rich membrane structures, with size ranging from 0.5 to few ?m, that form close contact with the surrounding substrate. Podosomes are more dynamic and instable than focal adhesions, dissolving and then reforming in new locations. They typically appear as a ring of adhesive molecules centred around a dense F-actin core [12]. It is still uncertain whether the first event in the formation of podosomes is actin nucleation or adhesion to the extracellular matrix (which leads to the activity of scaffolding proteins made up of combinations of protein/lipid and protein/protein interaction motifs involved in podosome formation and turnover). Furthermore, circular superstructures of podosomes, called rosettes, are formed spontaneously or after the stimulation with growth factors. The formation of podosomes and sealing zones as primary adhesive structures of resorbing osteoclasts upon contact with bone is well documented, but podosomes can be formed on a variety of other substrates. Interestingly, they appear as functioning-dependent mechanosensors [8]. Thus, they are sensitive to changes in substrate rigidity [9] and influenced by cellular contractility. Podosomes represent a powerful paradigm to study the mechanosensory machinery that integrates cytoskeletal elements with adhesion and signalling.

3 Modelling hyperstructures

3.1 HSIM

The stochastic automaton, HSIM, has been used to show that PTS and glycolytic metabolons can increase production of pyruvate eightfold at low concentrations of phosphoenolpyruvate [1]. A fourfold increase in the numbers of enzyme EI led to a 40% increase in pyruvate production, similar to that observed in vivo in the presence of glucose. Surprisingly, little improvement resulted from the assembly of metabolons into a hyperstructure. However, HSIM allowed us to see that such assembly is a powerful way of generating gradients of metabolites and signalling molecules. During the last year, we have been using HSIM to investigate how pausing during the replication of

the chromosome might lead to differential gene expression [25]. Although the situation analysed was somewhat different from the one we envisage, it is encouraging that there is a report of replication-associated changes in the expression of the dps, pyrI and gapA genes [2].

3.2 BioDyne

BioDyn is a multiagent software made to simulate self-assembly systems at cellular level. An agent is a cube located in a 3D grid. An agent can move according to the three axes (x,y and z) with a certain probability per simulation step. To allow self-assembly, two kinds of interactions are modelled. A short-distance adhesion interaction allows the agents to aggregate and form large rigid structures like actin filaments. Adhesion is given a probability which allows structures to be reconfigurable. A medium-distance attraction/repulsion interaction is designed to reproduce the action of 'soft' interactions which lead, for example, to the formation of the mitochondrial network. In order to improve the number of biological mechanisms that BioDyn can simulate, a software engine of reactions between agents has been made. It can reproduce phenomena like agent proliferation, agent destruction, agent creation or agent modification. The combination of the interactions and the reaction engine makes possible, in an abstract and qualitative manner, the reproduction of different biological hyperstructures. This system has different drawbacks. For instance, it is impossible to rotation an agent (only translations are allowed) and the multiscale is limited to one order of magnitude. BioDyn is available as a java applet at the url http://netbiodyn.tuxfamily.org. Example and tutorials are also available online.

3.3 Interaction networks

Interaction networks can prove useful in modelling hyperstructures. Indeed, a hyperstructure can be modelled by means of an interaction network $R = (N, I, f)$ which is defined as follows:

- N is a set of nodes representing the molecules, macromolecules and ions which, by associating physically, constitute the hyperstructure;
- I is the set of arcs and hyperarcs representing, respectively, the simple interactions (involving two or more elements of N, i.e. two molecules, macromolecules or ions) and the multiple interactions (involving more than two elements of N);
- f is an interaction function defined on the set I and whose the values could represent properties of the interactions (for example their intensities).

Such a representation would allow the simulation (e.g. via a Multi-Agent System) of processes involving hyperstructures such as the formation of a hyperstructure, the interactions between its constituents, or the interaction function related to it, etc.

Let us consider the example of the compaction hyperstructure (for references see [24]. In *E. coli*, the MukB protein is localized to discrete structures, with reports suggesting that it forms either foci at the [1/4] and [3/4] positions during the cell cycle or larger oblongs in the nucleoid. In vitro, MukB is associated with MukE and MukF in a large complex. MukB is a member of the SMC superfamily (like RecN), while MukF is a non-SMC protein or kleisin. It is generally believed that the MukB, MukE, and MukF proteins form a "condensin" that compacts DNA, probably in association with DNA gyrase, and that this condensin assists in the separation of sister chromosomes. With such a role in chromosome topology, it is not surprising that the MukB and SeqA foci are related. Indeed, the latter are perturbed in both size and distribution in the mukB null mutant. An interaction network of the type $R = (N, I, f)$ could then be used to obtain an interaction network $R' = (N', I', f')$ modelling a compaction hyperstructure; this would have a node set N = { condensing(MukB, MukE, MukF); chromosomes; DNA gyrase; DNA}, and a interaction set I which express the interactions respectively between condensing(MukB, MukE, MukF) and DNA gyrase and DNA, and between condensing(MukB, MukE, MukF) and chromosomes. The interaction network R' would have a node set I' = {sister chromosomes, complex(Mukb, SeqA foci), compacted DNA}.

3.4 Functioning-dependent structures

The concept of functioning-dependent structure (FDS) describes an assembly of objects that forms and maintains itself as a result of its action in accomplishing a task and that dissociates when its task is over. It is therefore a scale-free concept. To explore quantitatively this concept at the level of macromolecules, we have studied the behavior in steady state of a simple model of the functioning-dependent association of two sequential enzymes of the Michaelis-Menten type (Thellier et al., 2006). We are now studying the properties of an FDS in transient states using the MAPLE 9.5 software to solve the set of ordinary non-linear differential equations that constitute the model. Our initial findings are that a two-enzyme FDS can have properties that are very different from those of a system of free enzymes. The most salient of these properties is the generation of bursts of the final product. We suggest that, in principle, almost any metabolic pathway in which the enzymes can associate in a functioning-dependent manner can generate signals.

Some of the hyperstructures mentioned above fall into the class of functioning-dependent structures. This is the case for the pdu hyperstructures which disappear when the substrate, 1,2-propanediol, is removed and which cannot be purified in the absence of 1,2-propanediol. Remarkably, pdu hyperstructures do not form in vivo in the absence of 1,2-propanediol even under conditions where the genes are induced (Tom Bobik, personal communication). This may also be the case of the RNA degradosome (see above) where the association between RNase E, Hfq (an RNA binding protein) and sRNA (small, non-coding regulatory RNA) may also depend on the binding of the substrate. The sRNA act by base pairing with mRNA targets to inhibit translation and promote mRNA degradation. In *E. coli*, Hfq is required for the function of sRNA and RNase E has a role in the degradation of mRNA targets. Note that sRNAs are not present in normally growing cells; they are induced by a 'stress' or 'shock', accumulating to relatively high concentration for a transient period. Since researchers in the field have failed to detect a direct protein-protein interaction between Hfq and RNase E, one model for the interaction is that the sRNA is acting as a 'bridge' between Hfq and RNase E. The evidence suggests that sRNA induction leads to remodeling in which the canonical RNA degradsome is 'transformed' into a complex containing RNase E, Hfq and sRNA (for references see [6]).

3.5 Globally Constrained Shape Deformation

Jean-Marc Delosme has been working on the deformation of shapes under constraints on both perimeter and area which occur during the dynamics of hyperstructures such as the Golgi. Modelling these deformations is a challenging task due to the highly non-trivial interaction between the need for flexible local rules for manipulating the boundary and the global constraints. In collaboration with Freddy Bruckstein and Ishay Goldin, he has developed several methods to address this problem that entail "random walks" in the space of shapes obeying time-varying constraints on their perimeter and area [13]. Design of perimeter and area preserving deformations are an interesting and useful special case of this problem. The resulting deformation models are employed in annealing processes that evolve original shapes toward shapes that are optimal in terms of boundary bending-energy or other functionals. Furthermore, such models may find applications in the analysis of sequences of real images of deforming objects obeying global constraints as building blocks for registration and tracking algorithms.

3.6 Hyperstructure interactions

A think-tank in the Epigenomics Project (a 'Nirvana') brought together physical chemists (Jerry Manning, Max Berkowitz and Camille Ripoll), a physicist specialising in water structures (Alfons Geiger), and biologists interested in hyperstructures (Frank Mayer, Francois Kepes and Vic Norris). The first objective was to relate the phenomenon of counterion condensation (whereby a fraction of the counterions will run along a linear, charged polymer provided that the number of charges per unit length exceeds a critical value) to water structures and to 2-D structures such as membrane domains [28, 18]. The second objective was to treat counterion condensation as a solution looking for a problem in biology and then to come up with that problem. There is an urgent need in systems biology for solutions to the problem of how cells negotiate the enormity of phenotype space and, in particular, of how cells generate the coherent and reproducible phenotypes on which natural selection acts. The think-tank therefore had the objective of exploring a possible contribution in terms of ion condensation. A significant advance was made when the regulation of the cell cycle of bacteria was identified as the area in which suitable biological problems might be found - and the nature of the key step in the initiation of chromosome replication was identified as the most promising fundamental problem to explore. A hypothesis was then formulated in which Initiation is controlled by an initiation hyperstructure in which:

1. The key event of the separation of the strands at the origin of replication is mediated in part by the DnaA protein. This separation is favoured by the decondensation of ions from the origin region which leads to the strands in the origin region repelling one another. Such movements of ions involve linear filaments, membrane domains and water structures.
2. Sequestration of newly replicated origins of replication is controlled by a sequestration hyperstructure. The formation of this hyperstructure is favoured by the recondensation of ions occurring as the GATC sequences in the origin DNA go from being fully methylated to being hemi-methylated so increasing the charge parameter. This condensation also stabilises polymers of the SeqA protein which binds to these sequences.
3. Sequestration ends when ions decondense from this DNA and from the SeqA polymers in the sequestration hyperstructure to condense elsewhere on other hyperstructures.
4. The fundamental nature of the cell cycle has its origins in the coupling between ion condensation and the growth of the cell in terms of the production of the linear polymers and membranes that constitute certain hyperstructures. When the mass of polymers in the form of, for example,

ribosomal hyperstructures, reaches a critical threshold, condensation on these polymers at the expense of condensation on origin DNA leads to the decondensation step in 1/ that triggers initiation.

Encouragingly, it was realised that this mechanism might also underlie a related phenomenon in *E. coli*, that of growth rate control in which there is regulation of the proportion of the bacterial mass in the form of the transcriptional and translational apparatus. The group also realised that ion condensation and water structures might play important roles in the separation of the chromosomes and in membrane domain formation at the start of cell division.

4 Experimental advances

4.1 DNA replication and glycolysis

The replication of DNA and the central metabolism of carbon have recently been shown to be related in *Bacillus subtilis* where the chain elongation step of replication can be modulated by the flux of carbon through the bottom part of the glycolytic pathway [14]. Our expectation is that hyperstructure dynamics would underpin this coupling. How might DNA replication hyperstructures and glycolytic hyperstructures communicate? The alarmone ppGpp is over-produced when translating ribosomes meet uncharged tRNAs or when carbon sources are depleted. Over-production of ppGpp is strongly implicated in the coupling between replication and translation when there is a dramatic shortage of amino acids [34]. Is ppGpp produced at different basal level in steady state cells grown in different media also involved in this coupling? Our preliminary results indicate that it is not involved.

4.2 Membrane cartography

A collaboration between microbiologists and chemists in Rouen is intended to provide information on the lipid preferences of abundant membrane proteins as a way to study those hyperstructures that affect the dynamics and composition of the *E. coli* membrane. Initial work has focussed on a thorough mass spectometry-based analysis of the lipids making up this membrane [26, 27]. This is the basis for future experiments designed to reveal the changes in lipid composition that accompany overproduction of particular membrane proteins.

5 Discussion

Extending the concept of functioning-dependent structures to hyperstructures raises some interesting questions. The synthesis of macromolecules (DNA,

RNA, proteins, glycans, polyphosphates) is an important part of what makes a cell a cell. There is now ample evidence that such synthesis occurs in hyperstructures such as the lac hyperstructure where 30 or so RNA polymerases work together to generate the nascent mRNAs that are translated by 300 or so ribosomes [16]. Perhaps the most important one, again in *E. coli*, would be the putative ribosomal hyperstructure bringing together rRNA genes, rprotein genes and their products in a giant assembly plant (like the eukaryotic nucleolus) [5] although this is controversial [17]. The question we should ask is 'what do our FDS studies tell us about the dynamics of hyperstructures'? But first, what do our studies tell us about the signalling properties inherent in the synthesis of macromolecules by a single RNA polymerase or ribosome?

At the level of the synthesis of an individual protein, there is a set of individual reactions that add amino acids to the growing peptide. An individual reaction is between the amino acid and the nascent peptide as catalysed by the codon and the tRNA. The separate reactions that must be linked to make a protein require the channelling that is provided by the ribosome. So we might suppose the synthesis of an individual protein is an extreme example of an FDS. In terms of signalling, we can ask about what happens when protein synthesis is interrupted. Are peptide fragments released as signals (for references see [11])? Are ribosomes released (perhaps post-translationally modified)? And what about GTP/GDP and alarmones such as ppGpp and Ap4A? Note that at certain times in bacteria, many proteins are made only to be degraded. We might ask similar questions about RNA synthesis ...

So how is the functioning-dependent lac hyperstructure different from the FDS for synthesizing a single protein? In terms of complete assembly and disassembly of the FDH, specific lipids normally bound to nascent proteins in the FDH might be released by disassembly (with accompanying changes in viscosity of the membrane). Assembly and disassembly might affect water structures, DNA supercoiling and ion condensation. So topological signals are probably greater for an FDH than for an FDS. More usefully for our modelling, when a ribosome meets an uncharged tRNA, what happens to the following ribosomes (and what would be the equivalent for RNA polymerases?) - maybe they are all released? In other words, is there an amplification of effects in an FDH?

Finally, the insight that the hyperstructure hypothesis offers into bacterial physiology via hyperstructures extends to pathogenesis. The degradosome plays a key role in the virulence of bacteria such as Yersinia pestis [36]. This virulence is extremely sensitive to levels of calcium and to temperature, factors that are important in ion condensation. As mentioned about, it may be significant that the degradosome exists in the form of the linear filaments on

which, conceivably, ions might condense to regulate virulence. In which case, targetting such hyperstructures via hybrid metabolites or hybolites may prove of value [22].

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