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Thermodynamics of the prokaryote nuclear zone

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Abstract: In studying the functional and evolutionary significance of compartmentation in biology, it is instructive to consider its thermodynamic context as a conceptual centrepiece of entropy and phase transitions. Here we focus specifically on compartmentation at the intracellular level of microbial organellar cytology. Via a colloid-statistical argument, supplemented with order of magnitude estimates for the relevant physical quantities, we find that the DNA-containing nucleoid of prokaryotes presents a plausible nucleation site for phase-transitional behaviour, provided the genome exceeds some threshold size of the order of 10 Mbp. Large genome size seems capable in this respect of seeding compartmentation effects such as the nuclear envelope of *Planctomycetes* bacteria, which is widely regarded as a possible precursor to the nuclear envelope of eukaryotes.
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The collective function of the million or so macromolecules expressed in a prokaryote is very much entwined with the extremely confined physical nature of the intracellular space. Thus at the DNA headquarters, the nucleoid, control of its shifting structure and segregation under transcription is thought to be largely down to the degree of 'crowding' by the surrounding macromolecules (Zimmerman & Murphy 1996; Odijk 1998; Lewis *et al.* 2000; Zimmerman 2006). Crowding is essentially a colloid-like statistical mechanical parameter, and hence it is instructive to view the nucleoid and the wider prokaryote cytoplasm against the backdrop of classical high-concentration colloidal behaviour such as phase separation and the glass transition (Walter & Brooks 1995; Sear & Cuesta 2003; Braun & Bergenholtz 2007). Phase separation, in particular, has attracted attention because it in effect constitutes a route to subcellular compartmentation. Compartments formed in this way might afford a way for the organism to achieve a short-term localization of components for biochemical purposes (Long *et al.* 2005). Over the longer time scale of cytological evolution, they might serve as precursors to the emergence of fully fledged membrane-bound organelles (Braun *et al.* 2005; Fuerst 2006).

The theoretical treatment of phase separation is clearly rather formidable in a system of the complexity of prokaryotic cytoplasm, comprising as it does a heterogeneous mixture of very many components. However, in the wake of advances in predicting phase behaviour for general complex fluid mixtures (reviewed by Sollich (2002)), some mileage may be gained by focusing only on low moments of the weight distribution of cytoplasmic components. This 'method of moments' approach seems to confirm that phase separation is indeed broadly feasible in typical prokaryote cells, via the mechanism of so-called spinodal decomposition (Braun *et al.* 2005). The decomposition process begins with spontaneous amplification of long wavelength concentration undulations, followed by coarsening and coalescence. It is a 'bulk' instability, in that it can proceed in principle without the aid of a surface. However, should a surface of some kind be to hand, then this is where the latter stages of the process will tend to focus, where the nascent compartment will be seen to emerge. The concave inner surface of the cell membrane is perhaps the most obvious candidate in this respect. However, the DNA nucleoid itself presents an interesting further possibility. This is the scenario we wish to address here.

Consider the general situation for heterogenous nucleation on a spherical surface, radius R_0 (for a review of nucleation in protein solutions, see Sear (2005)). We denote by $\Delta f\,(<0)$ the thermodynamic free energy density favouring the nascent phase, such that the total free energy cost of enveloping the sphere in a compartment of radius R is

$$F = \frac{4\pi}{3}\Delta f(R^3 - R_0^3) + 4\pi(\gamma R^2 + \gamma_0 R_0^2). \tag{1}$$

Here γ_0 and γ denote the interfacial tensions at the inner and outer perimeters of the compartment, respectively. As usual in thermodynamics, the system strives to lower its free energy. Hence compartment nucleation and growth must satisfy a criterion $(dF/dR)_{R_0} < 0$. This translates to a criterion $R_0 > R_c = -2\gamma/\Delta f$ on the curvature radius of the surface.

It is reasonably sound to write, on dimensional grounds, $\gamma = kT/l_m^2$ for the interfacial tension, where kT is the thermal energy and l_m is a characteristic macromolecule length scale.

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Table 1. *Physical parameters of bacterial cytoplasm relevant to compartment nucleation at the level of the second virial approximation (for an overview of these estimates (apart from the virial coefficient itself), see Goodsell (1991)). Note for this combination of parameters that* $\partial\mu/\partial\rho = NkT/V + 2BkT(N/V)^2 < 0$*, so the cytoplasm is spinodally unstable. The discussion does not apply otherwise*

Total expressed macromolecules	N	10^6
Total intracellular volume	V	$1\ \mu m^3$
Macromolecule volume fraction	φ	30%
Macromolecule length scale	l_m	10 nm
DNA base pair length scale	l_{bp}	1 nm
Second virial coefficient for cytoplasm (Braun 2006)	B	$-10^{-6}\ \mu m^3$

Moreover, if we assume that the nucleoid is in a compact condensed state, then its effective radius scales with the size of the genome L (in base pair units) roughly as $R_0 = l_{bp}L^{1/3}$, where l_{bp} is a characteristic base pair length scale. Hence we can recast our criterion on R_0 with respect to genome size,

$$L_c = \left(\frac{-2kT}{l_m^2 l_{bp}\Delta f}\right)^3. \tag{2}$$

Estimates for l_{bp} and l_m are given in Table 1. It remains to somehow estimate Δf. Previous work (Braun *et al.* 2005) suggests in this regard that cytoplasmic compartmentation is closely tied to a well-known generic liquid–liquid (L–L) transition already present in the phase diagram of mono-disperse attractive colloids (including various proteins studied *in vitro*, as reviewed by Thurston (2007)). That is, compartmentation can be viewed as primarily a dressed-up colloidal L–L phase separation, accompanied, in a secondary modulating role, by partitioning of the protein inventory. Now the basic thermodynamics of the L–L transition is adequately described by a simple second-order virial expansion in particle number density $\rho = N/V$: each particle experiences a chemical potential $\mu/kT = \ln\rho + 2B\rho$, where B is the second virial coefficient, and when $\partial\mu/\partial\rho < 0$ they undergo spinodal decomposition. The associated nucleation free energy density is $\Delta f/kT = \rho^2\partial_\rho(\mu/kT)(\delta\varphi/\varphi)^2 = (\rho + 2B\rho^2)\ (\delta\varphi/\varphi)^2$, where $\varphi + \delta\varphi$ is the volume fraction in the nascent phase relative to φ in the parent phase. Hence, with substitution into Eqn (2) along with the physical parameters from Table 1 and $\delta\varphi \sim 0.1$, we arrive at an order of magnitude estimate,

$$L_c \sim 10\,\text{Mbp}.$$

Albeit very crudely, this substantiates a connection between large genome size and compartmentation, as summarized graphically in Fig. 1. Is there any experimental evidence for such a connection? A very tentative yes is suggested by the *de facto* template for prokaryotic intracellular compartmentation, the so-called 'pirellulosome' of *Planctomycetes* bacteria (Fuerst 2006). The pirellulosome is the only

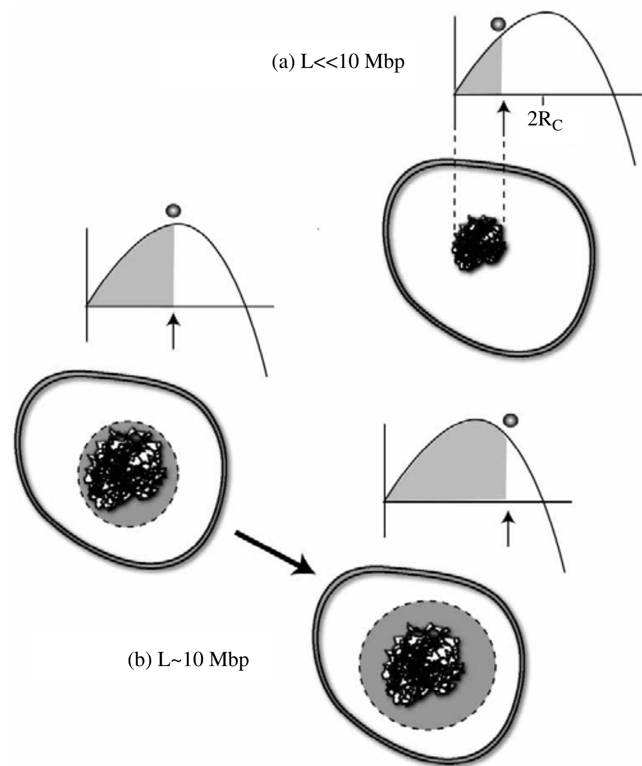

Fig. 1. The trend of Eqn (1) with the diameter is maximized at some critical value $2R_c$. If the DNA nucleoid is much smaller than this, as in (a), then it is ineffective as a nucleation site for the nascent compartment. The larger nucleoid of (b), on the other hand, serves to alleviate the free energy barrier, rendering growth of the compartment thermodynamically favourable.

well-characterized example of a membrane-bound nuclear envelope in extant prokaryotes. Although a universally shared cytological feature within the *Planctomycetes* phylum; nothing comparable is seen in any other prokaryote species. Currently there are four *Planctomycetes* genome sequences to hand, finished or nearly so. They weigh in at 6.6 Mbp *Blastopirellula marina*; 7.14 Mbp *Rhodopirellula baltica*; 7.8 Mbp *Planctomycetes maris*; 9.2 Mbp *Gemmata obscuriglobus*; these numbers rank them among the largest prokaryote genomes sequenced to date. (By way of comparison, the model bacterial genome *E. coli K12* has 4.6 Mbp.)

How do we qualify those similarly large prokaryote genomes not associated with intracellular compartmentation? Here it is pertinent to bring into the picture the issue of osmoregulation. Our naive virial expansion formulation already admits an osmoregulatory scenario in which the organism actively suppresses phase separation via uptake of cytoplasmic water and/or a global reduction in expression levels (since $\partial\mu/\partial\rho$ is driven positive by increasing V and/or decreasing N). Yet clearly such drastic measures could irredeemably disrupt system-biological coordination within the

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cell. A more prudent alternative strategy might be the uptake and circulation of compatible osmolytes. This proposition remains speculative (Braun 2006), but it has an interesting corollary: in an 'osmotically primitive' prokaryote lineage lacking this facility, compartmentation would be inevitable once the genome hits L_c; so this is a sort of accident waiting to happen[1].

A complete theory of how the *Planctomycetes* pirellulosome came about in its entirety will of course require much more – not least some account of its membrane encasing. The thermodynamic considerations outlined here seem to contribute an important skeletal fragment to the overall puzzle, but certainly there are many other factors outstanding, including those which are best approached within the disciplinary paradigm of cell and molecular biology. As ever in attempting to reduce biology to an idealized physical sketch, the devil's in the detail.

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[1] It is worth remarking that, in contrast to other large bacterial genomes, the provisionally available *Planctomycetes* genomes mentioned in the text reveal scant evidence of compatible osmolyte (CO) uptake systems (annotation is still in progress, however). CO-uptake generally involves the ABC superfamily ('ATP-binding cassette') of transporters (Lucht & Bremer 1994). For a given genome, a list of genes annotated as ABC subunits can be obtained from the NCBI protein database using the search query '(organism name) osmoregulation'. The list typically includes genes either specifically annotated as CO-binding subunits, or showing significant BLAST homology to genes with this annotation in other genomes.

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