Journal of Microbiological Methods 84 (2011) 147–150

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Perspective paper

How much cytoplasm can a bacterial genome control?

J.T. Trevors [a,*], L. Masson [b,1,*]

[a] School of Environmental Sciences, University of Guelph, 50 Stone Road, E., Guelph, Ontario, Canada N1G 2W1
[b] Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Ave., Montreal, Quebec, Canada H4P 2R2

ARTICLE INFO

Article history:
Received 3 November 2010
Accepted 7 November 2010
Available online 21 November 2010

Keywords:
Bacteria
Cell division
Cytoplasm
Genome
Synthetic microbiology
Energetics
Metabolism
Evolution
Cell size

ABSTRACT

In this perspective we discuss that bacterial genomes have optimized during evolution to control a range of cytoplasm, from immediately after cell division to a maximum amount/volume present just prior to DNA replication and subsequent cell division. The genetic expansion of bacteria *via* evolution may be limited to a genome size: cytoplasm amount/volume ratios and energetics that have been selected for during 3.6–4 billion years of evolution on the Earth. The optimal genome size is one that is relatively constant, but also has some plasticity for evolutionary change (*via* gene transfer) and mutational events, and can control a range of cytoplasm during the cell cycle.

1. Introduction

There are an immense number of unanswered questions central to understanding the origin of microbial life on the Earth and its subsequent evolution. For examples, how did microbial life and regulated cell division originate (Trevors, 2004)? What amounts of cytoplasm and cell sizes can small bacterial genomes control, or is there a genome:cytoplasm ratio that reaches a maximum just before cell division?

DNA has become the primary information macromolecule, elegant in its structure, paradoxically simple, and yet complex in its composition and capability of replication. It is the repository for organic, genetic information in all members of the three major kingdoms of life. Table 1 summarizes some relationships between bacterial genomes and the amount of cytoplasm controlled by a bacterial genome. The nature of these relationships may bring forth useful knowledge necessary in the construction of both synthetic and semi-synthetic microbial cells in future research and industrial applications. Table 2 contains minimal gene class functions in bacterial cells that would be necessary for cells to grow and divide regardless of the genome size, and genome to cytoplasm ratios. These core gene classes can also be hypothesized to have been necessary for the first bacterial cell(s) on the Earth to grow and divide and respond to natural selection events.

The genome replicates at least once so two identical genomes are present just prior to cell division. It is also known that some bacterial species have multiple copies of their genomes (see Table 1). Bacterial cells cannot commence DNA synthesis until they have sufficient cytoplasm for two identical cells. Bacterial cells are genetically programmed to divide. Virtually all metabolic activity is present to accomplish cell division under diverse and often rapidly changing environmental conditions. Since bacterial cells cannot foresee their future environments they can only respond *via* gene expression. Some other alternatives are cell death, spore formation in those species capable of forming spores, or entry into the physiological state of starvation survival until the environmental conditions change and available nutrients can be transported or diffuse into the bacterial cells. Different environmental conditions equate to different cellular global gene expression profiles geared towards cell division or sometimes in the case of spore-forming microorganisms, spore formation until sporulation can occur and then cell division. Even when analyzing a global gene expression event, this is never done on a single cell basis. Therefore, it is plausible that different cells in the sample may be expressing different genes, even in a balanced culture growing in a chemostat, with synchronized growth and cell division. It is known that bacterial cells are programmed to divide under a range of often diverse environmental conditions that do not exceed their minimal and maximum growth values (e.g., pH, temperature, available water, composition of gaseous environment, nutrient concentrations, toxicant concentrations, and antibiotic concentrations). Bacterial cells may divide because beyond a threshold cell size, bacterial cellular life is not possible (Caldwell, 1995). By dividing, the bacterial cell restores both the

* Corresponding authors. Tel.: +1 519 824 4120x53367; fax: +1 519 837 0442.
E-mail addresses: jtrevors@uoguelph.ca (J.T. Trevors), luke.masson@nrc-cnrc.gc.ca (L. Masson).
[1] Tel.: +1 514 496 3123; fax: +1 514 496 7251.

0167-7012/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2010.11.009

Table 1
Some possible relationships between bacterial genomes and amount of cytoplasm controlled by a bacterial genome. (Note: About 1051 bacterial genomes have been completely sequenced as of mid-2010).

- Genome is the genetic instruction set or ultimate blueprint of the cell that determines how a cell responds to a changing environment (Whitworth, 2008).
- Some bacteria have both a linear and circular chromosome (e.g., *Agrobacterium tumefaciens*) (Ochman, 2002).
- Some bacteria (e.g., *Epulopiscium* spp.) contain multiple copies of their genome (Mendell et al., 2008b).
- Sizes and organization of bacterial genomes vary; even strains of the same species can vary by as much as 20% in gene content (Boucher et al., 2001).
- A genome of 500–1000 kb occupies a large portion of the volume of a cell in the 150–250 nm diameter range (Trevors and Psenner, 2001).
- Genome:cytoplasm ratio is a product of natural selection for about 3.6 to 4 billion years.
- Bacterial genomes can only control an upper range of cytoplasm (and cell size) equivalent to the amount of cytoplasm in the cell just prior to cell division.
- For each species a genome to cell cytoplasm ratio is maintained within a range determined by evolution.
- Genome:cytoplasm ratio may be the ratio required for microscopic cells to overcome environmental entropy.
- Small bacterial genome sizes allow rapid cell division.
- Diversity in bacterial genome sizes is common.
- Smaller genomes may be the result of evolution by genome reduction from larger genomes.
- There are limits to bacterial genome expansion.
- Genome organization reflects the bacterial lifestyle.
- One strain's genome sequence is not entirely representative of other members of the same species.
- Smallest bacterial genome is 580 kb (*Mycoplasma genitalium*).
- Larger bacterial genomes are about 6300 kb (e.g., *Pseudomonas aeruginosa*).
- Bacteria can increase their survival in changing environments by altering their genetic instructions (via transformation, conjugation, transduction, transposition, mutations, deletions, and recombination).
- Bacteria with large genomes may have evolved by doubling of genome sizes during evolution.
- Plasmids are widely distributed in bacterial species.
- Bacterial cells maintain their cell-surface to cell-volume ratios within a range.
- Smaller bacterial genomes sizes may confer the selective advantage of shorter generation times (Ranea et al., 2005).
- Genome sizes during evolution may have been controlled in part, by restriction-modification systems' by protecting the host genome and a particular range for the genome size (Trevors, 1998).
- Bacterial cells must be large enough to carry out a range of integrated metabolic functions.
- Transport rates of some molecules into bacteria are a function in part of the surface areas of the cells.
- G + C content of bacteria ranges from 20 to 75% (Mann and Chen, 2009).
- Obligate intracellular bacteria have smaller genomes derived from free-living bacterial ancestors.
- Single genomes are incomplete representations of the actual total gene content of an entire bacterial species (Dobrindt and Hacker, 2001); (pan-gene, collection of genes shared among members of the same species; Tettelin et al., 2008; Lawrence and Hendrickson, 2005).
- Bacterial cells controlled by entirely synthetic DNA have been engineered (Gibson et al., 2010).

Table 2
Minimal gene class functions in bacterial cells.

Cofactor biosynthesis
Cell envelope
Cellular processes
Central metabolism
Energy production and storage
Lipid and fatty acids metabolism
Purine and pyrimidine metabolism
Regulatory functions
Replication, recombination and repair
Transcription
Translation
Transport of nutrients and elements
Some unknown genes-to be determined

normal cell size and the surface:volume ratio, and possibly genome to cytoplasm ratio.

It can be hypothesized that the cell requires a specific amount of genome to control a specific amount of cytoplasm and cell division restores this ratio, and the cell cycle (period from one cell division to the next) can then repeat if the conditions for growth are favorable. An interesting experiment would be different genome:cytoplasm ratios in a synthetic, semi-synthetic cell or even in natural cells where the amount of cytoplasm and genome are adjusted if this becomes experimentally possible. Parameters such as global gene expression, cell viability, cell size parameters, generation times and cell division could then be measured to obtain information on these parameters and the genome:cytoplasm ratios.

From a bacterial evolutionary perspective, the first bacterial genomes could be hypothesized to have controlled only a small amount of cytoplasm in small cell structures (probably in the nanocell range). However, the genome would need to contain all the necessary genes for controlled cell division (core or minimal genome size concept; Itaya, 1995) to prevent any loss of cytoplasm and the core or minimal genetic material. Even today, no single-celled living microorganism has all its genes understood in terms of their encoded functions (Gibson et al., 2010).

Mycoplasma genitalium has the smallest genome of any independently replicating prokaryotic species, synthesizing only 485 proteins. The advantages of a minimal core genome are minimal total metabolism, spatial economy and rapid cell division (Cavalier-Smith, 2005). Moreover, only 381 genes are necessary and this minimal set of essential genes is the subject of a patent by the J. Craig Venter Institute (Rockville, MA, USA). This knowledge points the way for synthesizing a minimal core chromosomal template to which functional genes modules (e.g., metabolic pathways for specific industrial applications) can be added to engineer microbial cell factories to produce targeted bioproducts like biofuels, industrial enzymes, novel end products or the complete degradation of specific pollutants.

The complete chemical synthesis of the *M. genitalium* genome has been completed containing all genes of the reference type with the exception of a disrupted antibiotic marker (Gibson et al., 2008). The synthetic genome was completely assembled in *Saccharomyces* cerevisiae *via* transformation-associated recombination cloning. These examples of DNA technologies applied to synthetic microbiology lay the foundations for the complete chemical synthesis of numerous genomes of increasing sizes. Recently, Gibson et al. (2010) reported on the construction of a bacterial cell (synthetic genome transplantation into *Mycoplasma capricolum*) controlled by a chemically synthesized genome. Moreover, combinations of synthetic and natural DNA segments should also be possible in the assembly of bacterial genomes. This type of research may bring forth important information on the amount of cell cytoplasm and the size of a bacterial cell that can be controlled by different bacterial genome sizes.

Another way to think of genome:cytoplasm ratios and corresponding cell sizes is that a cell with a diameter of about 156 nm could only accommodate about 250 genes or 250-kb of DNA while a cell with a diameter of 194 nm could contain a maximum of about 750 genes (Adams, 2001). Loferer-Kroßbacher et. al. (1998a, b) reported that bacterial cell diameters and genome sizes of some bacteria in ultraoligotrophic lake samples were as small as 0.2 μm with genomes of about 800 kb. A small bacterial cell with a small internal volume can only contain a correspondingly small amount of DNA compared to cells in the larger micron diameter range. Otherwise too much internal cell volume is occupied by DNA and there would be insufficient space for ribosomes and other cellular components. The small cell size however, has a large surface to volume ratio and diffusion distances for nutrients and gases are minimal. Such a small cell size with minimal diffusion distances would have been an excellent starting size for the origin of the first bacterial cells on the Earth. Small cells with a core genome and a small volume of cytoplasm are excellent structures for diffusion