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Perspective paper

How much cytoplasm can a bacterial genome control?

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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

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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

processes because of the surface area in contact with the surrounding environment, the short distance for diffusion across the first continuous cytoplasmic membrane and the concentration gradient from outside to inside the cells that would need to exist.

1.1. Bacterial genome to cytoplasm ratios: a possible experiment

An interesting experiment would be to estimate the amounts of cytoplasm synthesized in a known cell volume that is controlled by genomes of various sizes. Or conversely, how much cytoplasm volume is required for a range of genome sizes. It may be that synthetic microbiology holds the answers to these challenges. Bacteria ranging from nanobacterial dimensions (about 200 nm) to several microns in diameter, devoid of their normal genomes could be transplanted with synthetic genomes of varying sizes (Gibson et al., 2010) to determine if the cells remain viable and capable of growth and division. It would also need to be known if restriction systems were present that would restrict any DNA procedures for genome transplantation.

The capacity of the cytoplasm to process substrates is synchronized with membrane transport which is done by an optimal surface to volume ratio (in the order of 3:1 for some bacteria). Spherical cells have smaller surface area to volume ratios.

A large surface to volume ratio means that no internal part of the cell is distant from the cell surface where diffusion and transport mechanisms are in progress and nutrients are present. This allows a rapid generation time if the total environmental conditions are at the optimal or near optimal part of the growth range.

There are bacterial cells such as *Thiomargarita namibensis* that can reach a diameter of about 750 μm (Schulz and Jorgensen, 2001). This size should pose an immense diffusion problem as the normal high surface to volume ratio is decreased. The cell volume of such a large bacterial cell is in the order of hundreds of thousands of cubic microns. However, the volume is mostly occupied by a vacuole structure. *Thiomargarita* has evolved to overcome diffusion and surface to volume ratios obstacles by the presence of an immense centralized vacuole that occupies about 98% of the internal cell volume leaving the remaining cell volume for peripheral cytoplasm. Hence, the distances for diffusion processes are minimized and the genome to cytoplasm ratio is not affected because of the vacuole occupying the majority of the internal cell volume.

In all bacterial cells the mRNA following transcription must almost immediately locate the ribosomes for translation to proceed. In a large bacterial cell without cytoplasmic streaming how would this event occur in a suitable time domain?

In *T. namibensis*, the vacuole occupies an estimated 98% of the internal volume, meaning that the remaining 2% contains the DNA and ribosomes and the mRNA will contact the ribosomes without cytoplasmic streaming being necessary.

Another interesting structural situation occurs with large *Epulopsiscium pulopiscium* cells (600 μm and longer) which live in the guts of tropical fish (Mendell et al., 2008a). It is hypothesized that the presence of a nutrient rich environment in the gut compensates for the diffusion obstacle into large microbial cells. Moreover, this species reproduces by internal daughter cells whereby most of the DNA is arranged around the periphery of the cytoplasm. Large *Epulopiscium* cells contain an estimated 250 pg of DNA. This equates to several tens of thousands of copies of its average sized (about 3.8 megabase) genome. However, the genome to cytoplasm ratio may still be in the same magnitude as smaller micron sized bacterial cells, because of the large internal volume of the cell and corresponding cytoplasm amount, in relationship to the immense amount of DNA. This will need to be determined.

In a recent excellent and plausible hypothesis, Lane and Martin (2010) hypothesized that prokaryotic genome sizes are constrained by bioenergetics. To extend this hypothesis, the amount of cytoplasm controlled by the bacterial genome could also be constrained by bioenergetics. In eukaryotic cells the presence of mitochrondria permit an expanded number of genes to be present and expressed. Mitochrondria that produce ATP by oxidative phosphorylation have a core genome encoding proteins for the electron transport chain (Lane and Martin, 2010). This allows about a 200,000-fold increase in genome size in eukaryotic organisms compared to bacteria, were the cost of DNA replication is estimated at about 2% of the energy budget during growth, whereas protein synthesis accounts for 75% of the cells total energy budget (Lane and Martin, 2010). To expand the bacterial genome and protein synthesis would require an increased total energy budget. In addition, the metabolic cost of plasmid replication and transfer *via* conjugation (if the plasmid is conjugative) would also be a consideration in the energetic cost. The energetic cost of the plasmid can be reduced by simply not replicating the plasmid and its loss from the cell.

2. Summary

The hypothesis that genomes of various sizes can only exert genetic control over the amount of cytoplasm within a yet to be determined ratio is plausible. However, the actual experiments are still required. For example, *Escherichia coli* with a cytoplasm volume of about 0.67 μm^3 and about 4290 genes (with over 1000 unknown gene functions) would have a genome (gene number) ratio of about 6403 genes per 1 μm^3 of cytoplasm (using 1 μm^3 as a standard volume) in a cell where the DNA was not being replicated just prior to cell division.

The design, chemical synthesis/assembly of numerous synthetic bacterial chromosomes are technological challenges that are already being solved (Gibson et al., 2010). With synthetic biology emerging as a leading discipline in biological research, an important question/challenge will be to determine the amount of cytoplasm specific bacterial genomes or species (and other microorganisms) can control and the maximum genome size that can function in a precisely known amount and internal cell volume of cytoplasm. The concept of a genome to cytoplasm ratio may be useful in the emerging field of synthetic microbiology research.

Bacterial genome sizes and the corresponding amounts of cytoplasm may be limited by bioenergetics; the absence of mitochrodrial genes and metabolic oxidative phosphorylation capacity. The bioenergetics hypothesis of Lane and Martin (2010) is highly plausible.

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References

Adams, M.W.W., 2001. The Influence of Environment and Metabolic Capacity on the Size of a Microorganism. Size Limits of Very Small microorganisms. National Academy of Sciences, Washington, DC, USA.

Boucher, Y., Nesbo, C.L., Dolittle, W.F., 2001. Microbial genomes: dealing with diversity. Curr. Opin. Microbiol. 4, 285–289.

Caldwell, D.R., 1995. Microbial Physiology and Metabolism. Wm. C. Brown Publishers, Dubuque, IA, USA.

Cavalier-Smith, T., 2005. Economy, speed and size matters: evolutionary forces driving nuclear genome miniaturization and expansion. Ann. Bot. 95, 147–175.

Dobrindt, U., Hacker, J., 2001. Whole genome plasticity in pathogenic bacteria. Curr. Opin. Microbiol. 4, 550–557.

Gibson, D.G., Benders, G.A., Andrews-Pfannkoch, C., Denisova, E.A., baden-Tillson, H., Zaveri, J., Stockwell, T.B., Brownley, A., Thomas, D.W., Algire, M.A., Merryman, C., Young, L., Noskov, V.N., Glass, J.I., Venter, J.C., Hutchinson III, C.A., Smith, H.O., 2008. Complete chemical synthesis, assembly and cloning of a *Mycoplasma* genome. Sci. Express 319, 1215–1220.

Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.-Y., Algire, M.A., Benders, G.A., Montague, M.G., Ma, L., Moodie, M.N., Merryman, C., Vashee, S., Krishnakumar, R., Assad-Garcia, N., Andres-Pfannkoch, C., Denisova, E.A., Young, L., Qi, Z.-Q., Segall-Shapiro, T.H., Calvey, C.C., Pramar, P.P., Hutchinson III, C.A., Smith, H.O., Craig Venter, J., 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. Sci. Express. doi:10.1126/science. 1190719.

Itaya, M., 1995. An estimation of the minimal genome size required for life. FEBS Lett. 362, 257–260.
Lane, N., Martin, W., 2010. The energetics of genome complexity. Nature 467, 929–934.
Lawrence, J.G., Hendrickson, H., 2005. Genome evolution in bacteria: order beneath chaos. Curr. Opin. Microbiol. 8, 572–578.
Loeferer-Kroßbacher, M., Klima, J., Psenner, R., 1998a. Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis. Appl. Environ. Microbiol. 64, 688–694.
Lofere-Krioßbacher, M., Witzel, K.P., Psenner, R., 1998b. DNA content of aquatic bacteria measured by densitometric image analysis. Appl. Environ. Microbiol. 64, 688–694.
Mann, S., Chen, Phoebe, Y.-P., 2009. Bacterial genomic G + C composition-eliciting environmental adaptation. Genomics 95, 7–15.
Mendell, J.E., Clements, K.D., Choat, J.H., Angert, E.R., 2008a. Extreme polyploidy in a large bacterium. Proc. Natl Acad. Sci. USA 105 (18), 6730–6734.
Mendell, J.E., Clements, K.D., Choat, J.H., Angert, E.R., 2008b. Extreme polyploidy in a large bacterium. PNAS 105, 6730–6734.
Ochman, H., 2002. Bacterial evolution: chromosome arithmetic and geometry. Curr. Biol. R427–R428.
Ranea, J., Grant, A., Thorton, J.M., Orengo, C.A., 2005. Microeconomic principles explain an optimal genome size in bacteria. Trends Gen. 21, 21–25.
Schulz, N.H., Jorgensen, B.B., 2001. Big bacteria. Annu. Rev. Microbiol. 55, 105–137.
Tettelin, H., Riley, D., Cattuto, C., Medini, D., 2008. 12: 472–477. Comp. genomics bacterial pan genome. Curr. Opin. Microbiol. 12, 472–477.
Trevors, J.T., 1998. Molecular evolution in bacteria: genome size, cell size, restriction-modification and recognition. Bull. Inst. Pasteur 96, 25–33.
Trevors, J.T., 2004. Evolution of cell division. Theory Biosci. 123, 3–15.
Trevors, J.T., Psenner, R., 2001. From self-assembly of life to present-day bacteria: a possible role for nanocells. FEMS Microbiol. Rev. 25, 573–582.
Whitworth, D.E., 2008. Genomes and knowledge — a questionable relationship. Trends Microbiol. 16, 512–519.