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Chromosome dynamics in multichromosome bacteria

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Abstract

On the basis of limited information, bacteria were once assumed to have no more than one chromosome. In the era of genomics, it has become clear that some, like eukaryotes, have more than one chromosome. Multichromosome bacteria provide opportunities to investigate how split genomes emerged, whether the individual chromosomes communicate to coordinate their replication and segregation, and what selective advantages split genomes might provide. Our current knowledge of these topics comes mostly from studies in *Vibrio cholerae*, which has two chromosomes, chr1 and chr2. Chr1 carries out most of the house-keeping functions and is considered the main chromosome, whereas chr2 appears to have originated from a plasmid and has acquired genes of mostly unknown origin and function. Nevertheless, unlike plasmids, chr2 replicates once and only once per cell cycle, like a bona fide chromosome. The two chromosomes replicate and segregate using separate programs, unlike eukaryotic chromosomes. They terminate replication synchronously, suggesting that there might be communication between them. Replication of the chromosomes is affected by segregation genes but in a chromosome specific fashion, a new development in the field of DNA replication control. The split genome allows genome duplication to complete in less time and with fewer replication forks, which could be beneficial for genome maintenance during rapid growth, which is the norm for *V. cholerae* in broth cultures and in the human host. In the latter, the expression of chr2 genes increases preferentially. Studies of chromosome maintenance in multichromosomal bacteria, although in their infancy, are already broadening our view of chromosome biology.

Keywords

Divided genome; genome evolution; secondary chromosomes; genome maintenance; communication between chromosome replication and segregation; *V. cholerae*

Bacteria usually have one circular chromosome of a few megabases in size. They often have plasmids whose size range from a few to one hundred or more kilobases. In some cases, the plasmids approach the size of chromosomes. These are still called plasmids or megaplasmids because they are not always essential for cell viability. Owing to the development of genomics, it has become clear that bacteria can have more than one chromosome, each carrying essential genes. The emerging picture is that multichromosome bacteria have one primary chromosome (chr1) carrying most house keeping genes, and usually one or two secondary chromosomes with recognizable plasmid features and carrying some essential genes (1).

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Bacteria with multiple chromosomes have been found in diverse prokaryotic phyla including Actinobacteria, Chloroflexi, Deinococcus-Thermus, Fibrobacteres, Firmicutes, Proteobacteria (α -, β - and γ -classes), and Spirochaetes, suggesting that they have arisen independently, many times in the course of evolution. This is also evident from the fact that not all bacteria belonging to different classes of the same phylum have divided genomes. On the other hand, bacteria belonging to the same family, such as *Vibrionaceae*, almost always show divided genome (39/39 tested; (2)), indicating that once split, there is selective pressure to maintain the multichromosome state. As of this writing, about 10% of sequenced bacteria have split genomes. These bacteria are proving attractive systems to understand genome evolution, regulation of replication and segregation, and the basis of genome configuration. Most of our current knowledge on these topics in multichromosome bacteria has come from studies in *V. cholerae*, which has one primary (chr1) and one secondary chromosome (chr2).

Replication

The features of the replication origin of the primary chromosome of *V. cholerae* are essentially the same as those of the *Escherichia coli* chromosome (3, 4). In both bacteria, chromosome replication is controlled by the highly conserved initiator, DnaA, which has homology to the eukaryotic initiator complex ORC1-6 (5). Replication of chr2, on the other hand, is controlled by its specific initiator, RctB (6, 7). The genetic make-up of the region responsible for chr2 replication is similar to that of some *E. coli* plasmids, in having an array of repeats (iterons) in the origin of replication to which its specific initiator binds (Figure 1A). This similarity has prompted the suggestion that chr2 might have originated from a plasmid (3). At a detailed level, however, regulation of chr2 replication is more complex than those of iteron-carrying plasmids. Here, RctB binds not only to iterons but also a second kind of site, the 39-mers, which are the powerful negative regulators of chr2 replication (8). The activity of 39-mers is restrained by several mechanisms: transcriptional inactivation, in which transcription across a 39-mer site (in *rctA*, Figure 1A) interferes with RctB binding to that site; DNA-looping in which RctB bound to a 39-mer interacts with another RctB bound to an iteron; and competitive inhibition of RctB binding by RNA polymerase due to overlapping binding sites (*PrctB* and a 39-mer like site, Figure 1A). Restraining 39-mer action apparently is needed for replication initiation. RctB binding to iterons in the origin, which is essential for replication initiation, is also regulated by methylation of the sites, the extent of which varies during the cell cycle (8). The presence of two kinds of initiator binding site and some of the additional layers of control over the initiator binding are unprecedented in plasmids, and indicate that chr2 replication is controlled more stringently than that of plasmids.

Plasmid and chromosomal replication differ in a fundamental way. In general, chromosomal replication is restricted to a particular phase of the cell cycle, whereas plasmid replication can take place at almost any time of the cell cycle (9). Chr2 is a *bona fide* chromosome in this respect (10). How the additional layers of control found in chr2 might change the timing of replication from random to specific remains a subject of speculation (8).

In *V. cholerae*, chr2 is one-third the size of chr1 and initiates replication much later than chr1. This is presumably the reason why the two terminate replication roughly at the same time (10). This is the only indication at the moment that the chromosomes might be communicating with each other to coordinate their replication.

Segregation

Barring a few exceptions, which include *E. coli*, most bacteria carry *parAB* genes in their chromosome that participate in chromosome segregation. The genes were initially found in

many low copy number plasmids, and were shown to facilitate their segregation into daughter cells and thereby improve plasmid retention in growing bacterial cultures. The two chromosomes of *V. cholerae* have their own *parAB* genes, which function in a chromosome specific manner. These genes are largely homologous but the differences suffice to confer specificity (11). Similarly, the three chromosomes of *Burkholderia cenocepacia* and a carried plasmid all possess highly homologous, but replicon-specific, *parAB* genes (12).

Segregation of chr1 and chr2 of *V. cholerae* follows different paths. The chr1 origin of replication duplicates at one cell pole. One of the sister origins stays at the place of birth and the other moves to the opposite pole (11). This program is also characteristic of the single chromosomes of *Caulobacter crescentus* and sporulating *Bacillus subtilis* (13, 14), and chr1 of multichromosome bacteria *Agrobacterium tumefaciens* and *Sinorhizobium meliloti* (15). Chr2, on the other hand, initiates duplication at the cell center, and the sister origins move towards cell quarter positions. This program is also used by *E. coli* and vegetatively growing *B. subtilis* (16, 17). *V. cholerae* thus maintains two distinct chromosome segregation programs in the same cell. This might have been necessary to avoid competition between the chromosomes for space.

Equivalents of the mitotic spindle and motor proteins that segregate eukaryotic chromosomes have not been found in bacteria, and the basis of directional movement of sister chromosomes in bacteria remains largely unknown. Some Par proteins appear to be able to pull chromosomes at the final stages of chromosomal segregation (11, 14), but several other motive forces could be helping the process along the way. These include the acts of DNA replication and transcription, DNA condensation, linking chromosomes to growing cell membrane, and entropic exclusion (14). Much remains to be discovered about the mechanics of chromosome segregation in bacteria.

Repair of broken replication forks or other damage by homologous recombination between sisters can result in formation of chromosome dimers. The dimers need to be resolved into monomers before the sister chromosomes can segregate into incipient daughter cells. The same triumvirate (XerC, XerD and FtsK) resolves the dimers of both the chromosomes of *V. cholerae* (18).

Communication between replication and segregation

Although replication and segregation are the two main mechanisms that maintain chromosomes, they function mostly independently of each other. As mentioned earlier, the act of replication conceivably could move the newly replicated chromosomal branches away from each other, although this remains to be demonstrated. The converse process, segregation influencing replication, was unexpected but has been observed. Recently, the segregation protein, ParA, was found to control replication initiation of *B. subtilis* chromosome and *V. cholerae* chr1 by interacting with DnaA (19–21). In *V. cholerae*, ParB protein of chr2 also was found to promote chr2 replication by abrogating the activity of an inhibitory site (22). In *B. subtilis*, ParB helps to load SMC (condensin) proteins at the origin of replication, apparently to organize the origin region for replication and segregation (23, 24). These studies are broadening of our view of chromosome maintenance in bacteria.

Advantages of divided genome

Selective amplification of one of the chromosomes under certain environmental conditions can change gene abundance and thereby the levels of gene expression. Like other bacteria capable of fast growth under nutrient rich environment, *V. cholerae* selectively amplifies origin-proximal DNA of chr1 (25). The higher gene dosage allows more product formation from the origin-proximal region of the chromosome. Indeed, genes that are particularly

required during rapid growth, such as ribosomal RNA genes, are found close to the origin (26). During fast growth, the cell generation time can be much lower than the time taken to replicate and segregate chromosome. This potentially chaotic situation is managed by maintaining overlapping replication cycles, which increases the number of replication forks per chromosome (25, 27, 28). Longer chromosomes require more extended elongation period and more forks to keep up with cell growth. Dividing the genome and simultaneously replicating the constituent chromosomes not only reduces the total time to duplicate the genome but also completes replication with fewer forks (9). This is desirable for chromosome stability since forks are vulnerable to breakage during chromosome movement in segregation.

Selective elimination of chromosomes has also been suggested to have redeeming features. In a community of cells, such as in biofilms, selective chromosome elimination will generate cells that, although inviable, might still be able to provide their neighboring cells with certain gene products or provide structural support (as in Myxobacterial fruiting bodies), which could be advantageous to the community as a whole (3). However, *chrII* loss leads to degradation of *chrI* (29). Even cells with only *chrII* may not be of much use without *chrI* providing much of the transcription and translation machineries.

An interesting aspect of gene expression in *V. cholerae* is that when the bacterium was grown mid-exponentially in rabbit ileal loops, it showed expression of many more genes of *chr2* than those expressed in aerobically grown cells in rich medium and harvested at the mid-exponential phase (30). The results were similar when the bacteria were collected from stools of cholera patients (31). Limitation iron and oxygen appear to be the primary stresses in the rabbit intestine but how they selectively affect *chr2* remains to be understood.

Concluding remarks

The two chromosomes of *V. cholerae* are maintained by separate mechanisms for both replication and segregation, unlike the situation in eukaryotic cells, where the two mechanisms are common for all the chromosomes. The bacterial life style has most likely been selected to avoid competition among the chromosomes, which is apparently avoided in eukaryotes by making the relevant factors in excess. Another difference from eukaryotes is that although bacteria with more than one chromosome are common, bacterial chromosomes with multiple origins are not. In contrast, Archeal chromosomes have shown more than one active origin, although their size is similar to that of single-origin bacterial chromosomes (32). From these initial studies it appears that maintenance of multiple chromosomes in bacteria and in eukaryotes may not follow similar principles.

Studies of *V. cholerae* *chr2* have provided a fresh perspective on how chromosome replication can be timed in the cell cycle (8), a new example of transcriptional interference in protein (RctB)-DNA interactions in bacteria (Figure 1B) (33), and a target (RctB) for novel antibiotics specific to Vibrios, since this protein is not found outside of the *Vibrionaceae* family (34). If *chrI* communicates with *chr2* to synchronize termination of replication, this might reveal a novel check-point control mechanism. Since, *V. cholerae* maintains two distinct replication and segregation programs in the same cell, this should help distinguish between global and specific factors in the regulation of these processes. The parking spaces of the two chromosomes are largely separate. Entropic exclusion should not only apply between the two sisters of the same chromosome but also between the two chromosomes (35). Figuring out how the two chromosomes carve out their territories inside the crowded cell interior and how that affects their function are new challenges in bacterial cell biology. Finally, multichromosome bacteria are close enough to their monochromosome

counterparts to encourage experimental and conceptual cross-talk that should enrich our understanding of both.

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Highlights

- Some bacteria, like eukaryotes, have multiple chromosomes
- Some of these chromosomes appear to be of plasmid provenance
- Maintenance mechanisms of individual chromosomes are distinct, not as in eukaryotes

Figure 1.

A) Comparison between the regions responsible for replication of an iteron-carrying plasmid P1 of *E. coli* and chr2 of *V. cholerae*. Both the regions are divided into three functional units: *ori* (*oriP1/ori2*) that is required in cis to initiate replication, an autorepressed initiator gene (*repA/rctB*), and *inc* (*incA/incII*) that regulates replication. The *ori* has iterons (arrowheads) where the initiator binds, a site(s) for binding the universal bacterial initiator DnaA (green pentagons), and GATC sequences (red dots), whose adenine residue is methylated. The chrII region has more features: Its iterons have internal GATC sites and methylation of the sites are required for RctB binding (36), its *inc2* has a second kind of RctB binding site, called 39-mers (black rectangles) (8), and it has an ORF (*rctA*), whose transcription is also repressed by RctB (33). B) *rctA* might help to reduce fluctuation of the RctB level. If RctB level overshoots, the level can be adjusted down by reducing new synthesis because of tighter auto repression. At high RctB, *rctA* promoter will also be better repressed and, without transcriptional interference, titration can happen, which should reduce available RctB. Similarly, if the RctB level drops, the level can be restored by new synthesis and less titration because on-going transcription of *rctA* would interfere with RctB binding (33).