Trends in Microbiology

CellPress

Review

Hard-Wired Control of Bacterial Processes by Chromosomal Gene Location

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Bacterial processes, such as stress responses and cell differentiation, are controlled at many different levels. While some factors, such as transcriptional regulation, are well appreciated, the importance of chromosomal gene location is often underestimated or even completely neglected. A combination of environmental parameters and the chromosomal location of a gene determine how many copies of its DNA are present at a given time during the cell cycle. Here, we review bacterial processes that rely, completely or partially, on the chromosomal location of involved genes and their fluctuating copy numbers. Special attention will be given to the several different ways in which these copy-number fluctuations can be used for bacterial cell fate determination or coordination of interdependent processes in a bacterial cell.

How Genome Organization and Gene Function Are Connected

For decades, the importance of genome organization has been recognized. Virtually every process that interacts directly or indirectly with the chromosome has left its marks during the course of genome evolution. It has become clear that the order and orientation of features on a chromosome, as well as the three-dimensional structure of the chromosome, is of importance to a cell. Numerous examples of the interplay between genome organization and cellular processes are available. For example, essential genes tend to be located on the strand that is transcribed in the same direction as in which replication proceeds [1].

However, the importance of the genomic location of key elements is still often underestimated. In fact, very little attention is given to the many different ways in which genomic location can impact cell biology. We therefore review the various mechanisms by which the exact genomic location of a feature can play a role in the regulatory landscape and development of bacterial cells. More specifically, we focus on processes in which gene copy number or, more accurately, genome-wide copy number distributions play a role. It is a well established fact in eukaryotes that having an abnormal number of chromosomes (aneuploidy), leading to atypical gene copy numbers, can have detrimental effects, a well known example being Down syndrome (trisomy 21 in humans, [2]). Additionally, the need for female mammals to silence one of their two copies of the X-chromosome underlines the importance of DNA copy numbers [3,4]. Furthermore, amplification of specific nutrient transporter genes in *Saccharomyces cerevisiae* was observed to enhance fitness in nitrogen-limited conditions [5]. The correlation between copy number and gene expression implied by these examples was confirmed recently by Chen and Zhang, who showed that the timing of replication of a gene influences its final expression level in yeast [6]. Nevertheless, copy number effects are still only rarely considered in prokaryotes. During bacterial cell cycle progression, copy numbers around the chromosome fluctuate periodically. Both the periodicity [7] and the amplitude [8,9] of this fluctuation can be employed to regulate certain

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While well appreciated in eukaryotes, the relevance of gene copy numbers in bacteria is largely unexplored.

Copy number imbalance between two genes during DNA replication was found to be responsible for precisely timed activity pulses of the *Bacillus subtilis* sporulation initiator.

Besides the various ways in which chromosomal gene location has been shown to be involved in *B. subtilis* sporulation, more examples have recently been found in other bacteria as well.

Changing nutrient availability or malfunction of critical subcellular processes lead to distorted gene copy number distributions, with transcriptomic shifts as a result, for example accounting for the activation of pneumococcal competence.

Additionally, the discovery that mRNA diffusibility is unexpectedly low suggests that the chromosomal location of a gene may be in part determined by where in the cell its product is required.

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processes in the cell. Furthermore, global or local (e.g., compartmentalization, see below) distortions of copy number fluctuations can be involved in bacterial 'decision making' and even play an important role during virulence [9].

Replication-Associated Copy Number Fluctuations

The majority of bacteria have their DNA organized on a single, circular chromosome, replication of which starts at a well defined origin of replication (*oriC*). From there, replication proceeds symmetrically in both directions around the chromosome and is terminated at the opposite end (the *ter* region) of the molecule, where both replication machineries (forks) meet. As a result, the various genes and other features on the chromosome are replicated in a fixed order, leading to periodic fluctuations of their copy numbers that are repeated every cell cycle. After termination of replication, cells still need a specific amount of time to finish cell division (the D-period [8]). The initiation of new rounds of replication is tightly regulated by a variety of factors [10–13]; this ensures that there is exactly one initiation event in each cell cycle, timed in such a way that replication and cell division are properly coordinated. When growth is sufficiently slow, cells have enough time to start and finish DNA replication within one cycle, and local copy numbers will generally only fluctuate between one and two copies of a certain region (Figure 1A). Some bacteria, however, have the capacity to grow so fast that replication of their entire chromosome cannot be completed within one cell cycle [14]. In this case, cells engage in multifork replication; before a replication fork has finished, a new replication initiation event takes place (still exactly once per cell cycle) at all ($\geq$2) copies of *oriC* simultaneously, resulting in copy numbers of *oriC*-proximal regions of more than 2 (Figure 1B). For example, fast-growing *Escherichia coli* cells have been observed to contain up to 8 origins [15]. Since there is a clear correlation between gene copy number and gene expression [16–18], these fluctuations are relevant to a cell's transcriptome, as is exemplified by the various cases mentioned in this review.

Function-Associated Gene Order

The amplitude of a gene's copy number fluctuation will thus depend both on its genomic location, relative to *oriC*, and on growth rate. The impact of these dependencies is illustrated by the fact that translocations and chromosomal inversions preferentially occur in a copy-number-neutral fashion (i.e., symmetrical with respect to *oriC*) [19–21]. Another example of the importance of gene order is the strong conservation of the *oriC*-proximal colocalization of important growth factors involved in replication, transcription and translation [14,22,23]. The colocalization of these factors can be explained by a combination of the importance of their stoichiometry on the one hand and functional compartmentalization (see below) on the other. However, the fact that they are virtually always found close to the origin of replication rather reflects the cells' need to correlate their expression with their requirement; when growth conditions improve, cells may switch to multifork replication, automatically boosting the expression of these essential growth factors due to the resulting dosage increase. Recent work by Soler-Bistué *et al*. demonstrates the relevance of the genomic position of ribosomal protein genes on the large chromosome of the human pathogen *Vibrio cholerae*, which harbors two circular chromosomes (Figure 1C) [9]. They showed that translocation of a locus bearing half of all ribosomal protein genes from *oriC*-proximal to various sites further away from the origin of replication results in significant defects in growth and host-invasion capacity. It is worth noting that these defects specifically occur during relatively fast growth, where the difference in copy number between *oriC* and *ter*, and therefore the relative effect of translocation of the ribosomal protein genes, is the largest. Both defects are relieved when, instead of one, two copies of the locus are present at an *oriC*-distal site, effectively restoring absolute ribosomal gene copy numbers and consequently ribosome production levels. The fact that these genes are then no longer colocalized with other important growth factors is, apparently, of lesser importance in this context.