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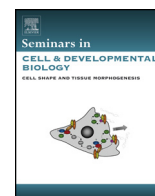
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## Review

Symmetries and asymmetries associated with non-random segregation of sister DNA strands in *Escherichia coli*

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## ABSTRACT

The successful inheritance of genetic information across generations is a complex process requiring replication of the genome and its faithful segregation into two daughter cells. At each replication cycle there is a risk that new DNA strands incorporate genetic changes caused by miscopying of parental information. By contrast the parental strands retain the original information. This raises the intriguing possibility that specific cell lineages might inherit “immortal” parental DNA strands via non-random segregation. If so, this requires an understanding of the mechanisms of non-random segregation. Here, we review several aspects of asymmetry in the very symmetrical cell, *Escherichia coli*, in the interest of exploring the potential basis for non-random segregation of leading- and lagging-strand replicated chromosome arms. These considerations lead us to propose a model for DNA replication that integrates chromosome segregation and genomic localisation with non-random strand segregation.

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## Contents

1. Introduction .....	611
2. Evidence for non-random DNA strand segregation .....	611
2.1. Early studies of DNA strand segregation .....	611
2.2. Non-random DNA strand segregation .....	611
2.2.1. The L-R-L-R configuration .....	611
2.2.2. Non-random DNA segregation of leading- and lagging-strands .....	611
3. Possible molecular mechanisms underlying non-random segregation .....	612
3.1. Asymmetries associated with DNA replication .....	612
3.1.1. Replisome structure and function .....	612
3.2. Asymmetric labelling of the leading- and lagging-strands during replication .....	613
3.2.1. Hemi-methylation of GATC sites .....	613
3.2.2. SSB coating of the lagging-strand .....	613
3.3. Genomic asymmetry between the right and the left replichores .....	613
3.3.1. Asymmetry of chromosomal macro-domains .....	613
3.3.2. Asymmetry of ribosomal RNA (rRNA) operons .....	613
3.3.3. Asymmetry of <i>oriC</i> segregation .....	613
3.4. Sequence asymmetry between the leading- and lagging-strands .....	614
3.4.1. GC skew .....	614
3.4.2. Gene density is higher on the leading-strand than on the lagging-strand .....	614
3.5. Asymmetric segregation of the nucleoid .....	614
4. Conclusion .....	616
Acknowledgements .....	616
References .....	616

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## 1. Introduction

The partitioning of cellular components between two daughter cells and subsequent cell division has been extensively studied in biology. The viability of daughter cells greatly depends on the accurate segregation of newly replicated chromosomes. In eukaryotic cells, chromosome segregation has been extensively studied from the nineteenth century and the fundamental steps and players in this process have been known from the 1960s [1]. In prokaryotic cells, the localisation of genomic DNA sequences within the cell, referred to here as genomic localisation, and chromosome segregation are closely linked. In *Escherichia coli*, genomic localisation has been well described [2,3]. However, chromosome segregation is less well understood. *E. coli* cells are rod shaped and divide by binary fission involving the formation of a septum at mid-cell. This means that a pair of newly formed cells is comprised of two outward-facing old-poles and two inward-facing new-poles. Recently, evidence has suggested that there is a strong bias for the replicated DNA strands to be segregated non-randomly to the daughter cells with the DNA copied on the leading-strands of the replication forks preferentially localised towards the cell poles and the DNA copied on the lagging-strands preferentially localised towards the mid-cell [4]. These authors proposed that this preference for a mirror-symmetrical disposition of DNA replicated on the leading- and lagging-strands underlies a preference for a translational symmetry of segregation of chromosomes [4]. Non-random segregation of DNA strands has the consequence that original “parental” DNA strands can be consistently segregated to specific cells in a lineage. In the case of *E. coli* the consequence of the preferentially observed segregation pattern is the co-localisation of the parental DNA strands with the poles of the first cells in the lineage (the “old” poles). Whether this has any evolutionary advantage is unknown. However, the non-random segregation of DNA strands in a multicellular eukaryote has the potential to maintain parental strands within a defined subpopulation (lineage) of stem cells with the clear advantage of limiting errors of copying. The proposal that a parental DNA strand is retained in a specific cell type has been called the “immortal strand” hypothesis [5].

Here, we review the evidence for non-random strand segregation in *E. coli* and its relationship to genomic localisation and chromosome segregation. Furthermore, we explore the asymmetries that may underlie non-random segregation and discuss a model that integrates chromosome segregation and genomic localisation with non-random strand segregation.

## 2. Evidence for non-random DNA strand segregation

Strong indications of non-random DNA strand segregation in *E. coli* have been gathered from the early 1970s to the present day. Several independent studies, all using very different approaches, were able to quantify the proportions of cells in which sister chromosomes segregate to generate particular patterns. These studies argue for a preferential segregation pattern observed in approximately 85% of events.

### 2.1. Early studies of DNA strand segregation

In the early 1960s, the replicon model for chromosome segregation proposed that cell growth could be responsible for chromosomal segregation in bacteria. For this proposition to be true it was suggested that (i) chromosomes are attached to and replicate at the cell membrane and that (ii) separation of the newly replicated chromosomes occurs by membrane growth between the two chromosomal attachment sites [6]. Supporting these hypotheses, a direct connection between the nucleoid and the cell membrane

was first reported in *Lactobacillus acidophilus* by Lark and collaborators [7]. Jacob and co-workers confirmed this result by studying the morphology of *E. coli* spheroplasts in which they were able to see a physical link between the membrane and the nucleoid [8]. Despite these studies, it remains unclear whether attachments to the cell membrane are implicated in chromosome segregation. A number of early studies investigating whether the segregation of DNA strands was random or non-random came to opposing conclusions. Several of these were interpreted as providing evidence for random segregation [8–11] and others for non-random segregation [12–15]. Of these early studies, work using labelling of new DNA strands with 5-bromodeoxyuridine (BrdU) followed by differential staining of nucleoids has provided strong evidence in favour of an “alternative segregation mechanism” in which directional segregation involving parental strands moving to the cell poles occurs in 60–80% of division events, while random segregation occurs in 20–40% of cell divisions (i.e. parental strands moving to the cell poles in 80–90% of events) [16].

Genetic screens designed to identify genes involved in chromosome segregation in *E. coli* have not helped to explain these segregation patterns. Most of the candidate genes are involved in chromosome catenation/decatenation, maintenance or control of cell shape and DNA repair processes. None of these contenders has yet been shown to have an obvious role in (non-random) chromosome segregation [17].

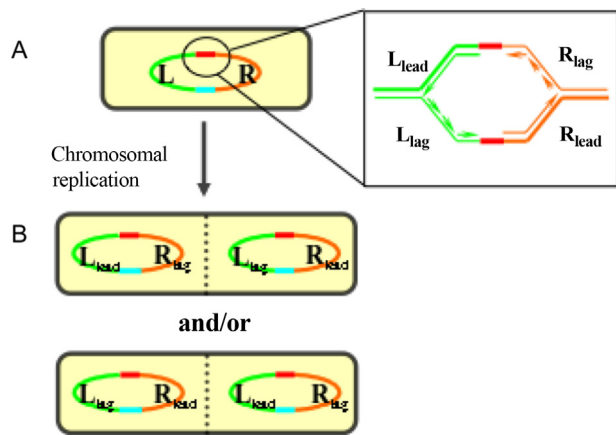
### 2.2. Non-random DNA strand segregation

#### 2.2.1. The L-R-L-R configuration

In *E. coli*, the initiation of DNA replication and cell division are not directly coupled, leading to the presence of multiple chromosome copies within a single, fast-growing, cell. This physiological particularity of *E. coli* complicates the analysis of chromosomal segregation. However, cytological techniques such as Fluorescent In Situ Hybridisation (FISH) and Fluorescent Repressor-Operator System (FROS), in combination with advances in microscopy in slow-growing cells, have allowed the localisation of different chromosomal loci within the cell. It has been shown that during the cell cycle *oriC* localises to the middle of the cell with each replichore (the left and right sides of the circular chromosome between *oriC* and the *ter* loci) located in a separate cell half (Fig. 1A). Consistent with this observation, the location of *ter* sequences was confirmed to cover a wide region between the two cell poles [2–4,18]. During replication, sister *oriC* sequences eventually migrate to the future mid-cell positions (in daughter cells) which correspond to  $\frac{1}{4}$  and  $\frac{3}{4}$  of the mother cell. This arrangement makes available a unique configuration for the rest of the chromosome in which each *oriC* sequence is flanked by left and right replichores [2,3]. If replication is initiated and finished before cell division, segregation in a cell with two chromosomes preferentially leads to a <Left-*oriC*-Right-Left-*oriC*-Right> (L-R-L-R) arrangement of replichores (Fig. 1A). This type of chromosome organisation is observed in 85% of the population analysed [3].

#### 2.2.2. Non-random DNA segregation of leading- and lagging-strands

The L-R-L-R configuration corresponds to a translational symmetry, which is most economically explained if leading- and lagging-strands segregate non-randomly. Either both leading-strands segregate to the poles of the cell while the lagging-strands segregate to mid-cell, vice versa or both patterns are allowed (Fig. 1B). In other words there is a mirror symmetry of the cellular distribution of leading and lagging segregated DNA strands [3,19]. Recently, White and co-workers used FROS, combined with an endonuclease-based system for distinguishing between leading and lagging strands of DNA replication, to determine the



**Fig. 1.** Chromosome organisation (A) and non-random DNA strand segregation in *E. coli* (B). The origin of replication domain is in red and the terminus of replication domain is in cyan. The chromosome is in green (left replichore: L) and orange (right replichore: R). The division septum is represented by a dotted line. (A) Origin and terminus domains are localised at mid-cell with left and right replichores in separate cell halves. Replication of leading- and lagging-strand templates is represented from the origin of replication domain. (B) Mirror symmetry of leading (lead) and lagging (lag) strands: lead-lag.lag-lead or lag-lead.lead-lag, explains the translational symmetry of sister chromosomes (L-R-L-R). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

segregation pattern of DNA strands. In a recombination deficient mutant ( $\Delta recA$ ), the lagging-replicated strand is degraded after induction of the endonuclease and, in 89% of cells, the remaining leading-replicated strand is segregated to a cell pole [19,20]. The authors were able to conclude that the leading-strand is preferentially segregated to the cell pole and inferred that the lagging-strand is preferentially segregated to mid-cell [19].

### 3. Possible molecular mechanisms underlying non-random segregation

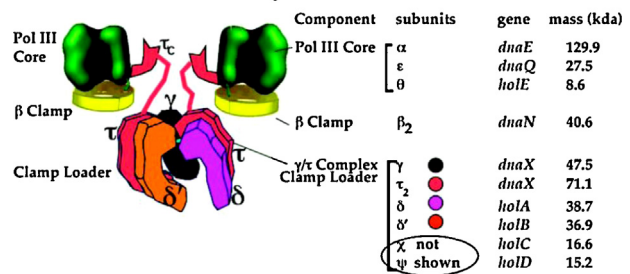
#### 3.1. Asymmetries associated with DNA replication

##### 3.1.1. Replisome structure and function

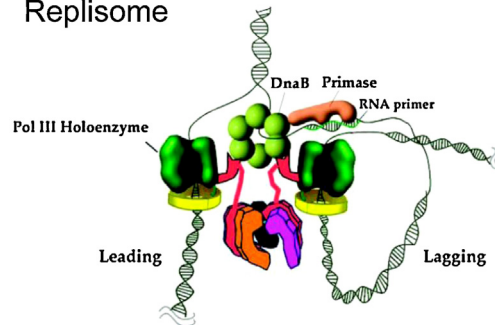
In *E. coli*, there are five different DNA polymerases. DNA polymerase III (Pol III) is the chromosomal replicase whereas DNA polymerase I is implicated in RNA primer removal on the lagging-strand. Both of these polymerases can also participate in DNA repair reactions. DNA polymerases II, IV and V are involved in DNA repair only. Two DNA polymerase III core enzymes, each bound to a  $\beta$  sliding clamp and held together by a  $\beta$  clamp loader, are classically considered to form the DNA polymerase III holoenzyme (Fig. 2A), but see below evidence for a third polymerase III subunit. Within the holoenzyme, the  $\beta$  clamp loader is formed by six subunits:  $\gamma$ ,  $2\tau$ ,  $\delta$ ,  $\delta'$ ,  $\chi$ . The  $\gamma$  and  $\tau$  subunits are encoded by the same gene (*dnaX*). However,  $\gamma$  has a 24 kDa shorter C-terminal region due to a truncation caused by a translational frameshift [21]. The  $\gamma$ ,  $\tau$ ,  $\delta$  and  $\delta'$  subunits are members of the AAA+ family of ATPases that are conventionally arranged in circles by homo-polymerisation. However, the  $\gamma$ ,  $\tau$ ,  $\delta$  and  $\delta'$  subunits of the  $\beta$  clamp loader form an open circle structure instead of the predictable closed circle, creating an asymmetric structure responsible for loading the  $\beta$  sliding clamps onto the DNA [21–23] (Fig. 2).

The holoenzyme functions in a structure called the replisome containing the hexameric DnaB helicase, the DnaG primase and single-strand binding protein (SSB). During replication, the replisome complex is very dynamic and protein interactions are fast

### A Pol III Holoenzyme



### B Replisome



**Fig. 2.** DNA polymerase III holoenzyme and replisome structure in *E. coli*. (A) Illustration of the protein arrangement within the holoenzyme. The subunit names for the core DNA polymerase III, the clamp and the clamp loader with their stoichiometry plus their coding gene and mass in kilodaltons are shown on the right hand side. (B) The asymmetric conformational structure of the replisome. The lagging-strand with the DnaB helicase and the DnaG primase has to make a loop in order to be replicated by the holoenzyme pol III.

Figure re-published with permission from [21]

and labile [21]. Using single molecule fluorescence microscopy, it was observed that there are actually three molecules of DNA polymerase per replisome [24]. This observation was confirmed and refined very recently as it was shown that there is polymerase exchange at the replication fork. Double labelling of DNA Pol III and SSB shows oscillations of these two molecules at the replisome corresponding to lagging-strand synthesis. This result suggests that a third polymerase subunit is present in the replisome and is exclusively involved in lagging-strand replication. This confers a structural asymmetry to the replisome [25]. The replisome also presents a conformational asymmetry caused by the antiparallel structure of the DNA. The polymerase working on the leading-strand replicates the DNA continuously, and in the same direction in which the parental DNA is unwound. However, the lagging-strand is replicated in the opposite direction. Consequently, the lagging-strand accommodates these opposed movements by forming a loop around the DNA Pol III holoenzyme on which specific proteins, DnaB, DnaG and SSB, act to replicate the chromosome (Fig. 2B) [21,26,27].

If these three independent levels of structural and conformational asymmetry of the replisome are responsible for the non-random segregation of the DNA strands, one might expect that the leading- and lagging-strand replicated genomic loci would be differentially localised immediately after the passage of the replisome. However, studies have shown that replicated sequences exhibit extensive post-replicative co-localisation and that release of this proximity depends primarily on the decatenase activity of Topoisomerase IV (TopoIV) [28,29] suggesting that the replicated sister strands remain topologically entwined for a significant period of time during which their “memory” of the asymmetric structure of the replisome might be lost.

### 3.2. Asymmetric labelling of the leading- and lagging-strands during replication

#### 3.2.1. Hemi-methylation of GATC sites

In *E. coli* cells, DNA is methylated by the DNA adenine methyltransferase (Dam methylase). It was suggested that Dam is associated with the DNA Pol III holoenzyme and scans the DNA for 5'-GATC-3' sites to methylate during DNA replication [30]. After passage of the replisome, and because of the semi-conservative replication of DNA, fully methylated GATC sites are converted into two hemi-methylated DNA sites. Methylation of hemi-methylated GATC sites by Dam is fast except in the *oriC* and the *dnaA* promoter regions. These regions are protected from Dam activity by the binding of SeqA [31]. During replication, the bulk of the two sister chromosomes is transiently labelled by hemi-methylated GATC sites. Because both copies of the two newly replicated chromosomes are hemi-methylated, it is difficult to conceive that hemi-methylation of GATC sites would be responsible for non-random chromosomal segregation in *E. coli*. Nonetheless, analysis of the origin of replication of ColE1 plasmids showed that *de-novo* methylation of hemi-methylated GATC sites on the leading-strand is twice as fast as on the lagging-strand (2 s versus 4 s). This suggests that methylation on the lagging-strand follows ligation of Okazaki fragments [32]. It also suggests that, if the methylation of GATC sites plays any role, it may not be the labelling per se but the dynamic sequence of reactions following replication that could have a role in non-random chromosome segregation.

#### 3.2.2. SSB coating of the lagging-strand

Single-strand binding protein (SSB) functions as a tetramer that cooperatively binds single-stranded DNA (ssDNA) with high affinity [33]. Through its C-terminal domain, SSB also defines the substrates upon which DNA replication, recombination and repair pathways must operate, providing an anchor-point for specific proteins [34]. During replication, SSB coats the lagging-strand that loops around the Pol III holoenzyme, creating an asymmetry between the leading- and the lagging-strands. In the replisome, SSB proteins are able to directly interact with the  $\chi$  and  $\psi$  subunits of the clamp loader of the holoenzyme and with the DnaG primase [35,36]. These two interactions are responsible for an exchange mechanism in which the primase dissociates from the RNA-DNA duplex and the clamp loader associates with the lagging-strand [36]. The asymmetric labelling of the lagging-strand with SSB and the subsequent interactions within the replisome are required for cell viability and accurate replication of the chromosome [37]. Since the C-terminus of SSB is critical for protein interactions, it could be a platform for proteins that specify the localisation of the lagging-strand chromosome. Large-scale proteomic studies using either dual affinity-tagged proteins [38] or hexa-histidine affinity-tagged variants of the *E. coli* proteome [39] have revealed several specific protein interactions with SSB [34]. One or more of these proteins could play a role in non-random chromosome segregation.

### 3.3. Genomic asymmetry between the right and the left replichores

#### 3.3.1. Asymmetry of chromosomal macro-domains

The chromosome of *E. coli* can be divided in two; the left and the right replichores defined by the bi-directionality of replication from origin to terminus. Furthermore, studies from Boccard and co-workers have refined the organisation of the chromosome to macro-domains within the two replichores. Six distinctive macro-domains have been defined: Ori, Ter, right region flanking Ter, left region flanking Ter and two less structured regions flanking the Ori macro-domain [40,41] and it has been proposed that they could influence chromosome segregation [40]. The localisation and

dynamics of each of the six macro-domains were analysed using fluorescence microscopy [42]. The results showed that the structured macro-domains (Ori, Ter, right region flanking Ter and left region flanking Ter) were less mobile than the rest of the chromosome. The authors also observed a sequential segregation pattern of loci on the two sister chromosomes that followed the order of genes from Ori to Ter on the chromosome. Overall, no difference was observed in the DNA segregation of the right and left regions flanking Ter. They concluded that each macro-domain defines a specific macro-space inside the cell.

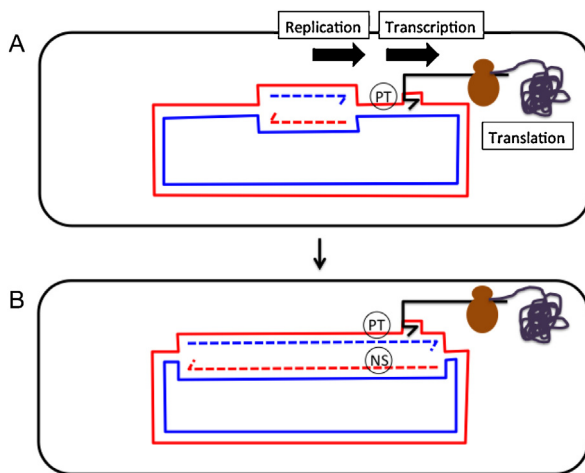
#### 3.3.2. Asymmetry of ribosomal RNA (rRNA) operons

There are seven rRNA operons in *E. coli*; each operon encodes a single transcript. Three rRNAs (16S, 23S and 5S) and at least one tRNA are the products of each operon. Transcription can start from two promoters, *rrn* P1 and *rrn* P2, and terminates at terminators t1 or t2 [43]. The seven operons are asymmetrically located on the chromosome and are all encoded on the leading-strand template [44]. Five of them are on the right replichore at positions 85', 87', 89.8', 90.7' and 4.8', whereas the two other are on the left replichore at positions 73.8' and 58.7' [45]. Noticeably, there is on the right replichore a cluster of four rRNA operons near the origin of replication. The reasons for the chromosomal location of the rRNA operons has constantly provoked curiosity [44]. One interesting feature of the rRNA operons is their regulation. Indeed, it has been shown that Fis and H-NS, two Nucleoid Associated Proteins (NAPs), bind and regulate in opposite ways the rRNA operons' activities. NAPs are known to be the most abundant chromosomal proteins and two roles have been attributed to them: (i) global gene regulation and (ii) nucleoid organisation [46,47]. rRNA operons could play a role in asymmetric segregation of the leading- and lagging-strands due to their high level of transcription on the leading-strand of the right replichore. If a mechanism exists that, following replication, favours transcription in the direction of replication from a replication template (the strand replicated on the leading-strand) to transcription from a newly synthesised strand (the strand replicated on the lagging-strand), then the unequal distribution of transcription between strands could translate into an unequal distribution of transcription between leading- and lagging-strand replicated chromosomes and a mechanism to distinguish between these strands [48] (Fig. 3).

#### 3.3.3. Asymmetry of *oriC* segregation

Two independent studies have followed the segregation of newly replicated *oriC* sequences using FISH techniques [18,42]. In the study from Bates and Kleckner, a two-stage segregation pattern for sister *oriC* sequences has been described including a specific asymmetry relative to which side of the nucleoid contains the *ter* locus. In slow growing conditions (generation time of 120 min), both origins remain close together near the middle of the cell immediately after replication starts. Then the *ter*-distal *oriC* locates to the  $\frac{3}{4}$  position of the cell whereas the sister *oriC* localises between the  $\frac{1}{2}$  and the  $\frac{1}{4}$  position of the cell for about 20 min. This *oriC* finally switches positions with *ter* to segregate at the expected  $\frac{1}{4}$  position of the cell whereas *ter* moves to the middle of the cell [18]. Controversially, Espeli and co-workers did not observe the same pattern of segregation. In their study, the sister Ori macro-domains segregate both to the  $\frac{1}{4}$  and  $\frac{3}{4}$  positions of the cell between DNA replication and cell division [42]. It is difficult to see how the asymmetric segregation of *oriC* sequences observed by Bates and Kleckner could relate to non-random DNA strand segregation, as both origins are associated with leading- and lagging-strands.

It has also been reported that a single interrupted 10 bp inverted repeat with a 5 bp spacer sequence acts as a positioning site for replicated *oriCs* in *E. coli*. This 25 bp sequence is called *migS* for migration site. It was found in screens for mutants defective in



**Fig. 3.** Potential for transcription of genes co-oriented with DNA replication to favour localisation of the leading-strand template towards the cell pole. (A) Bidirectional DNA replication is shown to have initiated but not to have progressed as far as a co-oriented gene that is being transcribed. Because of the polarity of DNA and the polarity of synthesis of RNA, transcription of a co-oriented gene must be from the parental DNA strand that is also the template for leading-strand synthesis in replication (PT). (B) DNA replication is shown to have passed the site of the co-oriented gene at which point there are two potential templates for transcription, the same original template strand for leading-strand DNA replication (PT) and the newly synthesised strand (NS). It has been proposed that a mechanism could exist that favours the continued use of the original template (PT) over the new strand (NS) for transcription (e.g. retention of use of the same physical strand or absence of strand interruptions in the parental strand) [48]. If this is so, then the DNA strand replicated as a leading-strand template is associated with a higher probability of being transcribed than the newly synthesised lagging-strand. Given that there is a bias towards co-orientation of highly transcribed genes with the direction of DNA replication, this predicts that the DNA double-strand resulting from leading-strand synthesis is more actively expressed than the DNA double-strand resulting from lagging-strand synthesis. If active gene expression favours localisation towards the cell pole where there is more space, this could provide a bias for the observed non-random segregation of DNA strands. In this figure the transcribed gene is shown to be translated as would be the case for most genes. However some highly transcribed genes (e.g. rRNA genes) will only be transcribed.

segregation of newly replicated *oriC* sequences [49,50]. The *migS* sequence is located in the right replicore, 211 kb away from *oriC*. It has been shown that *migS* function is independent of its location relative to *oriC* by introducing *migS* at different locations on the chromosome [50]. Non-random segregation could be achieved through a sequence asymmetrically located on the chromosome, as long as a mechanism exists for strand-specific molecular labelling that results in strand-specific asymmetric cellular localisation. Unfortunately, the *migS* site is localised symmetrically (midway between the cell pole and mid-cell) so is not a good candidate for conferring non-random segregation.

### 3.4. Sequence asymmetry between the leading- and lagging-strands

#### 3.4.1. GC skew

The proportions of the four nucleotides (A, C, G and T) are different on the templates of the leading- and lagging-strands in *E. coli*. It has been reported that there is a bias of GC content (also called GC skew) towards G on the leading-strand template [51]. This feature was confirmed by the complete sequencing of the *E. coli* genome where the authors found that the abundance of G (26.22%) on the leading-strands of both replichores is statistically higher ( $P < 0.001$ ) than the abundance of C (24.58%), A (24.52%) or T (24.69%) [52]. One of the causes of GC skew is a different mutation rate between the two strands, which is the result of the asymmetric DNA replication mechanism in *E. coli* (for details see [51,52]). In silico and wet experimental approaches have shown that motifs enriched in G

like KOPS (FtsK Orienting Polar Sequences) (5'-GGGNAGGG-3') and Chi (5'-GCTGGTGG-3') are over-represented on the leading-strand template [53,54].

Chi motifs play a key role in RecBCD dependent DNA double-strand break repair by homologous recombination in *E. coli*. Chi sites are responsible for the attenuation of the exonuclease activity of the RecBCD complex and the subsequent generation of 3' single-strand DNA overhangs, a key step in DNA double-strand break repair (for details of DNA break repair and recombination in *E. coli* see [55] and [56]). Chi sites are distributed throughout the entire chromosome and on average there is one Chi site every 4.5 kb [54].

The FtsK protein is a DNA translocase that links segregation of *ter* regions with cell division. FtsK molecules load onto chromosomal KOPS motifs and scan the newly replicated chromosomes in a polar manner converging at the termination region that includes the *dif* site-specific recombination site [53]. Subsequently, FtsK aligns sister *dif* loci, on each side of the division septum, allowing activation of the site-specific tyrosine recombinase XerD, which ensures that chromosome dimer resolution is effective [57]. KOPS motifs need to be frequent on the leading-strand and not too frequent on the lagging-strand to actively stimulate unidirectional translocation towards the *ter* region. An opposite distribution of KOPS motifs would be highly deleterious for the cell [58]. Because FtsK is only active during the late steps of the cell cycle (constriction of the septum at cell division), and is involved in the segregation of the *ter* region it is unlikely to be involved in other aspects of chromosome segregation [59].

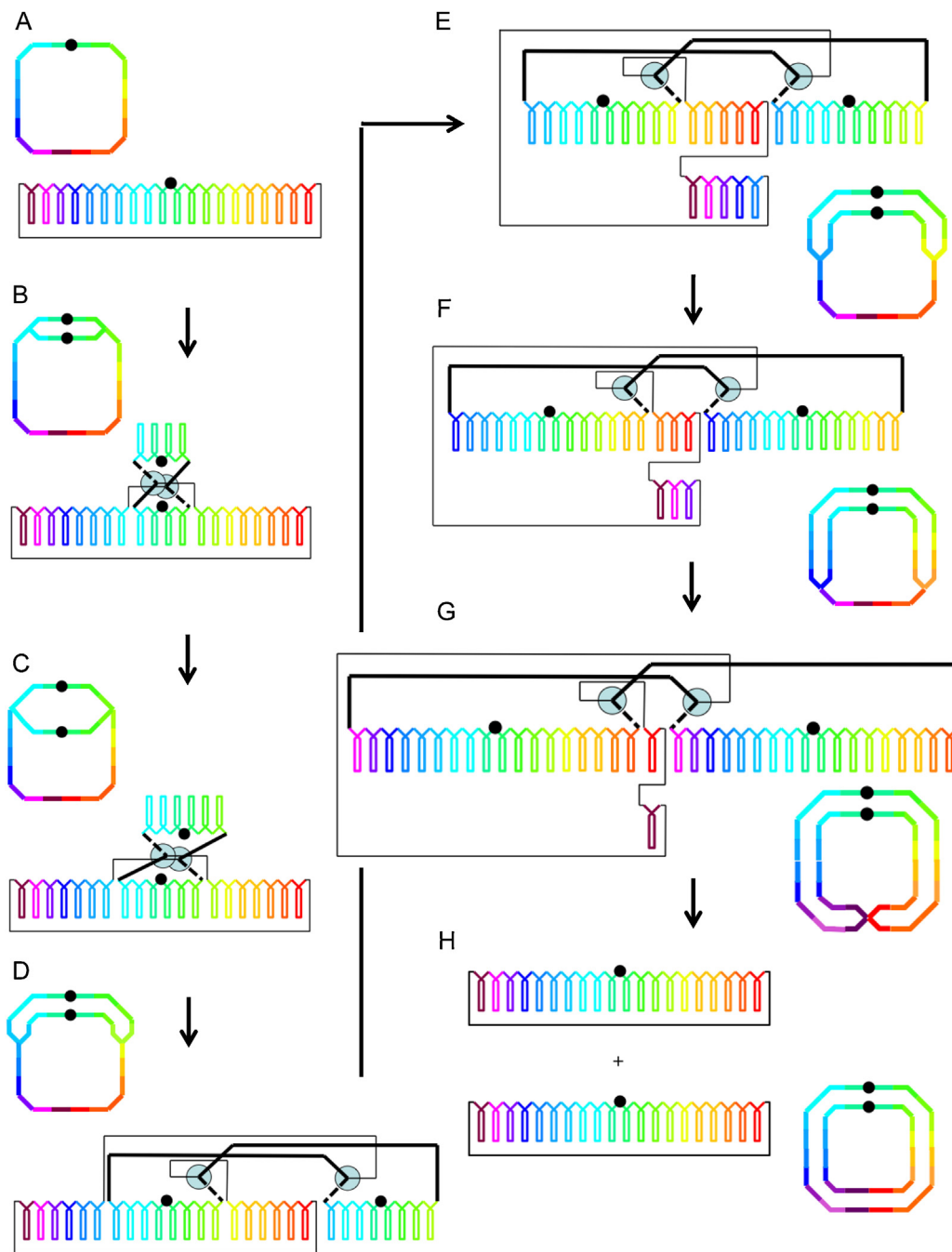
It is not clear how Chi or KOPS motifs (or indeed GC skew) could account for non-random chromosome segregation, unless these sequences could lead, in some unknown way, to differential labelling of the two sister chromosomes.

#### 3.4.2. Gene density is higher on the leading-strand than on the lagging-strand

The sequencing of many bacterial genomes has shown a general bias in favour of genes transcribed from the leading-strand template. In *E. coli*, 55% of genes are transcribed from the leading-strand template and 45% from the lagging-strand template [60,61]. It has been postulated that selection has favoured co-directional transcription and replisome progression [62]. Indeed, with the replisome travelling from *oriC* to *ter* and the RNA polymerase transcribing either in the same direction (from *oriC* to *ter*) on the leading-strand or in the opposite direction (from *ter* to *oriC*) on the lagging-strand, head-on collisions between the two polymerases can only happen if transcription occurs on the lagging-strand template. These collision events are understood to reduce the efficiency of replication [62,63]. The same mechanism suggested for the asymmetry of rRNA operons (Section 3.2.2) could operate at the whole genome scale. Indeed, different levels of transcription from leading- and lagging-strand replicated chromosomes could be a mechanism to distinguish between strands [48] (Fig. 3).

### 3.5. Asymmetric segregation of the nucleoid

The nucleoid is an extremely compact nucleoprotein complex relative to the total linear length of the chromosome, due to topoisomerase activities and protein attachment to the DNA [64,65]. Two recent studies have greatly enhanced our understanding of the segregation of loci along the length of the chromosome arms [66] and of the nucleoid structure and dynamics [67]. These studies have identified four nucleoid segregation transitions (T1–T4) of which T1 and T2 are asymmetric and correspond to the asymmetric two-stage segregation of the two newly replicated *oriC* sequences (see Section 3.3.3). Within the nucleoid, the movements of *oriC* sequences at



**Fig. 4.** Model for *E. coli* DNA replication that integrates chromosome segregation and genomic localisation with non-random strand segregation. This model reconciles the observed segregation of DNA strands with the L-R-L-R arrangement of replichores [2,3], key structures and transitions in the nucleoid [66] and the observed distribution of replisomes [68]. An interesting feature of the model is that the replisome is predicted to track the chromosome and remain located close to the DNA strand replicated on the lagging-strand. By contrast the leading DNA strand is translocated towards the cell pole. The chromosome is represented by rainbow colours and shown both as circular and linear structures. The linear structure represents the compacted structure of the nucleoid with markers on the left replicore being located in the left part of the nucleoid and markers on the right replicore being located in the right part of the nucleoid. The origin of DNA replication is represented as a black dot. (A) DNA replication has not yet initiated (the thin black line represents the connectivity of the circular chromosome). (B) DNA replication has initiated and a lateral bundle is formed that contains newly synthesised DNA. The new leading-stands are shown as continuous lines and the new lagging-strands as dotted lines (again the connectivity of unreplicated DNA sequences is represented by a thin black line). The replisomes are shown as blue circles. (C) DNA replication has continued and replicated markers have begun to move apart. (D) DNA replication has continued and an abrupt transition has occurred where the lateral bundle containing the newly replicated DNA moves to form a nucleoid bud. This corresponds to transition T1 where one origin is located at position  $\frac{3}{4}$  and one origin is located somewhere between  $\frac{1}{4}$  and  $\frac{1}{2}$  of the cell length [66]. (E) DNA replication has continued to the point where a second structural transition occurs and a region of terminal unreplicated DNA forms a lateral bundle that is centralised to allow the origins to become located at  $\frac{1}{4}$  and  $\frac{3}{4}$  of the cell length. This corresponds to transition T2 [66]. (F and G) DNA replication continues, absorbing the unreplicated lateral bundle. (H) DNA replication is complete and two nucleoids have been synthesised. Although represented here one above the other (for reasons of space on the page), they will in fact be arranged end to end in an L-R-L-R arrangement of replichores. The replisome can be seen to split into two foci that will separate from each other and then come back together, finally merging when the last part of the chromosome is synthesised as has been observed [68].

T1 may involve the release of tethers at *oriC*, whereas the movements of *oriC* and *ter* sequences at T2 are related to the release of tethers that maintain late cohesion at loci on the right replicore. Also, the T2 transition coincides with the appearance of an asymmetric bi-lobed nucleoid [66]. Two features of these reactions are asymmetric: the direction of movement of newly synthesised nucleoid with respect to the ends of the cell and the localisation of the tethers on the right arm of the chromosome. Furthermore, these studies have revealed an additional asymmetry. Loci on the right replicore are copied later than loci on the left replicore and segregate from each other correspondingly later, following a 7 min period of cohesion between the replicated sister chromosomes. The result of these asymmetric transitions at a macroscopic level is the appearance of a nucleoid bud on one side of the parental nucleoid that grows to form the new nucleoid. These observations suggest the presence of internal forces in the nucleoid that are responsible for the subsequent distribution and localisation of the newly replicated nucleoids prior to cell division.

#### 4. Conclusion

It is interesting to consider that the asymmetric L-R-L-R arrangement of replicores can be explained by a mirror symmetrical segregation of leading-strand replicated DNA to the cell poles and lagging-strand replicated DNA to mid-cell [19]. However, this does not explain the basis of the preference for a specific segregation pattern of replicated DNA strands. In this review, we have discussed several asymmetric processes that are known to occur in *E. coli* cells and that might underlie this segregation pattern. What seems to be required is a mechanism whereby some asymmetry is translated into a difference between the DNA strands replicated on the leading- and lagging-strands. Mechanisms such as the asymmetric structure of the replication fork, including the preferential binding of SSB to the lagging-strand template, are obvious candidates for distinguishing between strands. However, it is thought that the two replicated strands are held cohered for a period of several minutes following their replication [28]. If this is the case, they should remain cohered until the fork structure might have lost any influence on strand segregation. One hypothesis for sister chromosome cohesion is that it is caused by precatenanes that need to be removed by topoisomerase action [29]. If this were so, the mechanism for cohesion would not apply at the origin of replication since the precatenanes are generated from the relief of positive supercoils generated by DNA replication. Could it be that the direction of movement of leading- and lagging-strands is established very early, following the firing of the origin? Alternatively, could the direction of movement be established later, following the release of sister-strand cohesion? If so, some mechanisms that differentiate between the two sister strands must persist for a period of several minutes following DNA synthesis. This could be a sister chromosome-specific molecular label (e.g. a DNA modification mark), a sister chromosome-specific protein label (e.g. a protein preferentially attached to one or the other sister chromosome, delivered at the time of DNA replication) or a sister chromosome-specific activity (e.g. transcription being favoured on one or the other sister chromosome). Of all these possibilities only the last one has some support from previous work [48].

Whatever the mechanism for distinguishing the replicated DNA strands, the asymmetry of strand segregation needs to be understood within the context of the L-R-L-R arrangement of replicores and of the mechanism of segregation of the nucleoid. In an attempt to integrate the above features of chromosome organisation and segregation with DNA replication, we suggest a simplified model for these processes (Fig. 4). The model integrates the observations of Joshi and collaborators and Fisher and co-workers with

those of White and collaborators while maintaining the L-R-L-R arrangement of the replicores (see Section 2.2.1). We suggest that newly replicated DNA can form lateral bundles that can then move to extend the nucleoid. It is interesting to note that this model predicts that the replisomes track the chromosomes at the positions of the lagging-strand replicated sequences, leading to replisome separation and then convergence, as has been observed [68]. The model is simplified in several ways. Notably, Fisher and colleagues observed lateral bundling even in non-replicating cells, which means that shifting of DNA sequences between lateral arrays can presumably occur independently as well as during DNA synthesis. Furthermore, no specific reference is given here to the dynamic nature of the structures, including the longitudinal density waves observed or the tethering and tether breaking events that cause discontinuities in nucleoid segregation [67].

Are these observations and speculations relevant to other systems including the behaviour of eukaryotic chromosomes? At this stage, it is difficult to know. However, if non-random DNA strand segregation does occur, a mechanism of identifying strands is required and the direction of DNA replication through a critical part of the chromosome (e.g. the centromere) could be involved [69] leading to similar questions to those addressed in this review. It is not easy to imagine why *E. coli* might have evolved a replication system that preferentially segregates parental DNA strands in this way. However, in a multicellular eukaryote, the ability to determine the pattern of segregation of parental strands in such a way that an “immortal” DNA strand can be retained in a specific cell type may have important consequences within the context of differentiation, development and regeneration.

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#### References

- [1] Inoue S. Cell division and the mitotic spindle. *Journal of Cell Biology* 1981;91:131s–47s.
- [2] Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG. The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Molecular Microbiology* 2006;62:331–8.
- [3] Wang X, Liu X, Possoz C, Sherratt DJ. The two *Escherichia coli* chromosome arms locate to separate cell halves. *Genes and Development* 2006;20:1727–31.
- [4] Niki H, Yamaichi Y, Hiraga S. Dynamic organization of chromosomal DNA in *Escherichia coli*. *Genes and Development* 2000;14:212–23.
- [5] Cairns J. Mutation selection and the natural history of cancer. *Nature* 1975;255:197–200.
- [6] Jacob F, Brenner S, Cuzin F. On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symposia on Quantitative Biology* 1963;28:329–48.
- [7] Chai NC, Lark KG. Segregation of deoxyribonucleic acid in bacteria: association of the segregating unit with the cell envelope. *Journal of Bacteriology* 1967;94:415–21.
- [8] Ryter A, Hirota Y, Jacob F. DNA-membrane complex and nuclear segregation in bacteria. *Cold Spring Harbor Symposia on Quantitative Biology* 1968;33:669–76.
- [9] Chai NC, Lark KG. Cytological studies of deoxyribonucleic acid replication in *Escherichia coli* 15T-: replication at slow growth rates and after a shift-up into rich medium. *Journal of Bacteriology* 1970;104:401–9.
- [10] Lin EC, Hirota Y, Jacob F. On the process of cellular division in *Escherichia coli* VI. Use of a methocel-autoradiographic method for the study of cellular division in *Escherichia coli*. *Journal of Bacteriology* 1971;108:375–85.
- [11] MacQueen HA, Donachie WD. Intracellular localization and effects on cell division of a plasmid blocked in deoxyribonucleic acid replication. *Journal of Bacteriology* 1977;132:392–7.
- [12] Cooper S, Schwimmer M, Scanlon S. Probabilistic behavior of DNA segregation in *Escherichia coli*. *Journal of Bacteriology* 1978;134:60–5.
- [13] Cooper S, Weinberger M. Medium-dependent variation of deoxyribonucleic acid segregation in *Escherichia coli*. *Journal of Bacteriology* 1977;130:118–27.



- [14] Pierucci O, Helmstetter CE. Chromosome segregation in *Escherichia coli* B/r at various growth rates. *Journal of Bacteriology* 1976;128:708–16.
- [15] Pierucci O, Zuchowski C. Non-random segregation of DNA strands in *Escherichia coli* B-r. *Journal of Molecular Biology* 1973;80:477–503.
- [16] Canovas JL, Tresguerres EF, Yousif AM, Lopez-Saez JF, Navarrete MH. DNA segregation in *Escherichia coli* cells with 5-bromodeoxyuridine-substituted nucleotides. *Journal of Bacteriology* 1984;158:128–33.
- [17] Draper GC, Gober JW. Bacterial chromosome segregation. *Annual Review of Microbiology* 2002;56:567–97.
- [18] Bates D, Kleckner N. Chromosome and replisome dynamics in *E. coli*: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. *Cell* 2005;121:899–911.
- [19] White MA, Eykelenboom JK, Lopez-Vernaza MA, Wilson E, Leach DR. Non-random segregation of sister chromosomes in *Escherichia coli*. *Nature* 2008;455:1248–50.
- [20] Eykelenboom JK, Blackwood JK, Okely E, Leach DR. SbcCD causes a double-strand break at a DNA palindrome in the *Escherichia coli* chromosome. *Molecular Cell* 2008;29:644–51.
- [21] O'Donnell M. Replisome architecture and dynamics in *Escherichia coli*. *Journal of Biological Chemistry* 2006;281:10653–6.
- [22] Jeruzalimi D, O'Donnell M, Kuriyan J. Crystal structure of the processivity clamp loader gamma (gamma) complex of *E. coli* DNA polymerase III. *Cell* 2001;106:429–41.
- [23] Onrust R, Finkelstein J, Turner J, Naktinis V, O'Donnell M. Assembly of a chromosomal replication machine: two DNA polymerases, a clamp loader, and sliding clamps in one holoenzyme particle III. Interface between two polymerases and the clamp loader. *Journal of Biological Chemistry* 1995;270:13366–77.
- [24] Reyes-Lamothe R, Sherratt DJ, Leake MC. Stoichiometry and architecture of active DNA replication machinery in *Escherichia coli*. *Science* 2010;328:498–501.
- [25] Lia G, Michel B, Allemand JF. Polymerase exchange during Okazaki fragment synthesis observed in living cells. *Science* 2012;335:328–31.
- [26] Alberts BM, Barry J, Bedinger P, Formosa T, Jongeneel CV, Kreuzer KN. Studies on DNA replication in the bacteriophage T4 in vitro system. *Cold Spring Harbor Symposia on Quantitative Biology* 1983;47 Pt(2):655–68.
- [27] Yuzhakov A, Turner J, O'Donnell M. Replisome assembly reveals the basis for asymmetric function in leading and lagging strand replication. *Cell* 1996;86:877–86.
- [28] Lesterlin C, Gigant E, Boccard F, Espeli O. Sister chromatid interactions in bacteria revealed by a site-specific recombination assay. *EMBO Journal* 2012;31:3468–79.
- [29] Wang X, Reyes-Lamothe R, Sherratt DJ. Modulation of *Escherichia coli* sister chromosome cohesion by topoisomerase IV. *Genes and Development* 2008;22:2426–33.
- [30] Casades J, Low D. Epigenetic gene regulation in the bacterial world. *Microbiology and Molecular Biology Reviews* 2006;70:830–56.
- [31] Correnti J, Munster V, Chan T, Woude M. Dam-dependent phase variation of Ag43 in *Escherichia coli* is altered in a seqA mutant. *Molecular Microbiology* 2002;44:521–32.
- [32] Stancheva I, Koller T, Sogo JM. Asymmetry of Dam remethylation on the leading and lagging arms of plasmid replicative intermediates. *EMBO Journal* 1999;18:6542–51.
- [33] Chrysogelos S, Griffith J. *Escherichia coli* single-strand binding protein organizes single-stranded DNA in nucleosome-like units. *Proceedings of the National Academy of Sciences of the United States of America* 1982;79:5803–7.
- [34] Shereda RD, Kozlov AG, Lohman TM, Cox MM, Keck JL. SSB as an organizer/mobilizer of genome maintenance complexes. *Critical Reviews in Biochemistry and Molecular Biology* 2008;43:289–318.
- [35] Lu YB, Ratnakar PV, Mohanty BK, Bastia D. Direct physical interaction between DnaG primase and DnaB helicase of *Escherichia coli* is necessary for optimal synthesis of primer RNA. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:12902–7.
- [36] Yuzhakov A, Kelman Z, O'Donnell M. Trading places on DNA – a three-point switch underlies primer handoff from primase to the replicative DNA polymerase. *Cell* 1999;96:153–63.
- [37] Kelman Z, Yuzhakov A, Andjelkovic J, O'Donnell M. Devoted to the lagging strand-the subunit of DNA polymerase III holoenzyme contacts SSB to promote processive elongation and sliding clamp assembly. *EMBO Journal* 1998;17:2436–49.
- [38] Butland G, Peregrin-Alvarez JM, Li J, Yang W, Yang X, Canadian V, et al. Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* 2005;433:531–7.
- [39] Arifuzzaman M, Maeda M, Itoh A, Nishikata K, Takita C, Saito R, et al. Large-scale identification of protein-protein interaction of *Escherichia coli* K-12. *Genome Research* 2006;16:686–91.
- [40] Espeli O, Boccard F. Organization of the *Escherichia coli* chromosome into macrodomains and its possible functional implications. *Journal of Structural Biology* 2006;156:304–10.
- [41] Valens M, Penaud S, Rossignol M, Cornet F, Boccard F. Macrodomain organization of the *Escherichia coli* chromosome. *EMBO Journal* 2004;23:4330–41.
- [42] Espeli O, Mercier R, Boccard F. DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. *Molecular Microbiology* 2008;68:1418–27.
- [43] Paul BJ, Ross W, Gaal T, Gourse RL. rRNA transcription in *Escherichia coli*. *Annual Review of Genetics* 2004;38:749–70.
- [44] Nomura M, Morgan EA. Genetics of bacterial ribosomes. *Annual Review of Genetics* 1977;11:297–347.
- [45] Nomura M. Engineering of bacterial ribosomes: replacement of all seven *Escherichia coli* rRNA operons by a single plasmid-encoded operon. *Proceedings of the National Academy of Sciences of the United States of America* 1999;96:1820–2.
- [46] Dillon SC, Dorman CJ. Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nature Reviews Microbiology* 2010;8:185–95.
- [47] Vora T, Hottes AK, Tavazoie S. Protein occupancy landscape of a bacterial genome. *Molecular Cell* 2009;35:247–53.
- [48] Rocha EP, Fralick J, Vedyappan G, Danchin A, Norris V. A strand-specific model for chromosome segregation in bacteria. *Molecular Microbiology* 2003;49:895–903.
- [49] Fekete RA, Chatteraj DK. A cis-acting sequence involved in chromosome segregation in *Escherichia coli*. *Molecular Microbiology* 2005;55:175–83.
- [50] Yamaichi Y, Niki H. migS, a cis-acting site that affects bipolar positioning of *oriC* on the *Escherichia coli* chromosome. *EMBO Journal* 2004;23:221–33.
- [51] Lobry JR. Asymmetric substitution patterns in the two DNA strands of bacteria. *Molecular Biology and Evolution* 1996;13:660–5.
- [52] Blattner FR, Plunkett G, Bloch 3rd CA, Perna NT, Burland V, Riley M, et al. The complete genome sequence of *Escherichia coli* K-12. *Science* 1997;277:1453–62.
- [53] Bigot S, Saleh OA, Lesterlin C, Pages C, El Karoui M, Dennis C, et al. KOPS: DNA motifs that control *E. coli* chromosome segregation by orienting the FtsK translocase. *EMBO Journal* 2005;24:3770–80.
- [54] El Karoui M, Biauudet V, Schbath S, Gruss A. Characteristics of Chi distribution on different bacterial genomes. *Research in Microbiology* 1999;150:579–87.
- [55] Smith GR. How RecBCD enzyme and Chi promote DNA break repair and recombination: a molecular biologist's view. *Microbiology and Molecular Biology Reviews* 2012;76:217–28.
- [56] Michel B, Leach D. Homologous recombination—enzymes and pathways. In: Curtiss RI, Kaper JB, Squires CL, Karp PD, Neidhardt FC, Schlauch JM, editors. *EcoSal: Escherichia coli and Salmonella: cellular and molecular biology*. Washington, DC: ASM Press; 2012. <http://dx.doi.org/10.1128/ecosal727>.
- [57] Steiner WW, Kuempel PL. Cell division is required for resolution of dimer chromosomes at the dif locus of *Escherichia coli*. *Molecular Microbiology* 1998;27:257–68.
- [58] Touzain F, Petit MA, Schbath S, El Karoui M. DNA motifs that sculpt the bacterial chromosome. *Nature Reviews Microbiology* 2011;9:15–26.
- [59] Kennedy SP, Chevalier F, Barre FX. Delayed activation of Xer recombination at dif by FtsK during septum assembly in *Escherichia coli*. *Molecular Microbiology* 2008;68:1018–28.
- [60] Mao X, Zhang H, Yin Y, Xu Y. The percentage of bacterial genes on leading versus lagging strands is influenced by multiple balancing forces. *Nucleic Acids Research* 2012;40:8210–8.
- [61] Rocha EP, Guerdoux-Jamet P, Moszer I, Viari A, Danchin A. Implication of gene distribution in the bacterial chromosome for the bacterial cell factory. *Journal of Biotechnology* 2000;78:209–19.
- [62] Brewer BJ. When polymerases collide: replication and the transcriptional organization of the *E. coli* chromosome. *Cell* 1988;53:679–86.
- [63] French S. Consequences of replication fork movement through transcription units in vivo. *Science* 1992;258:1362–5.
- [64] Holmes VF, Cozzarelli NR. Closing the ring: links between SMC proteins and chromosome partitioning, condensation, and supercoiling. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97:1322–4.
- [65] Rocha EP. The organization of the bacterial genome. *Annual Review of Genetics* 2008;42:211–33.
- [66] Joshi MC, Bourniquel A, Fisher J, Ho BT, Magnan D, Kleckner N, et al. *Escherichia coli* sister chromosome separation includes an abrupt global transition with concomitant release of late-splitting intersister snaps. *Proceedings of the National Academy of Sciences of the United States of America* 2011;108:2765–70.
- [67] Fisher JK, Bourniquel A, Witz G, Weiner B, Prentiss M, Kleckner N. Four-dimensional imaging of *E. coli* nucleoid organization and dynamics in living cells. *Cell* 2013 [S0092-8674(13)00405-4].
- [68] Reyes-Lamothe R, Possoz C, Danilova O, Sherratt DJ. Independent positioning and action of *Escherichia coli* replisomes in live cells. *Cell* 2008;133:90–102.
- [69] Lew DJ, Burke DJ, Dutta A. The immortal strand hypothesis: how could it work? *Cell* 2008;133:21–3.