

THE REPLICATED *ftsQAZ* AND *minB*
CHROMOSOMAL REGIONS OF *ESCHERICHIA COLI*
SEGREGATE ON AVERAGE IN LINE WITH NUCLEOID
MOVEMENT

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3.1 Abstract

The average intracellular positions of the *ftsQAZ* region (2 min) and the *minB* region (26.5 min) during the cell cycle was determined by fluorescent *in situ* hybridisation using the position of *oriC* as a reference point. At the steady-state growth conditions used, newborn cells had replicated about 50% of the chromosome. By measuring the distances of the labelled *oriC*s with respect to mid-cell, we found two well-separated average *oriC* positions in cells of newborn length. These average *oriC* positions moved further apart along with cell elongation. The cellular

position of the *ftsZAZ* gene region resembled the position of *oriC*, although its average position was closer to mid-cell. In contrast, a single *minB* focus was observed at cell birth. Separated *minB* foci appeared towards the end of DNA replication. The average positions of *oriC*, *ftsZAZ* and *minB* relative to each other fitted a model in which DNA replication takes place in the cell centre and subsequent gene regions pass sequentially through this centre. We have interpreted the polarised orientation of the studied gene regions as a consequence of the mode of DNA segregation.

3.2 Introduction

Extensive cytometric analyses have demonstrated that *Escherichia coli* nucleoids move gradually, along with DNA replication and cell elongation (Van Helvoort and Woldringh, 1994). However, recent studies using light microscopic labelling techniques that visualise subnucleoid regions as fluorescent foci (Gordon *et al.*, 1997; Lin *et al.*, 1997; Webb *et al.*, 1997; Niki and Hiraga, 1998; Niki *et al.*, 2000) have indicated that such regions do not always segregate in line with the nucleoid. For instance, Gordon *et al.* (1997) found by time-lapse microscopy that, after duplication, one of the replicated origins visualised by the GFP-LacI/*lacO* system moved quickly away from the other. Movement of origins of replication has also been reconstructed from studies on fixed cells by fluorescent *in situ* hybridisation (FISH; Niki and Hiraga, 1998; Roos *et al.*, 1999; Niki *et al.*, 2000). The conclusions drawn by these authors diverge. Niki and Hiraga (1998) concluded that sister origins remain together near a cell pole for an appreciable time after duplication. Next, one of them moves quickly to the other pole. In contrast, we argue that such a conclusion cannot be drawn on the basis of FISH data if the position of the DNA replication cycle within the division cycle and the efficiency of FISH labelling are taken into account (Roos *et al.*, 1999; this paper). By performing FISH with DNA probes that hybridise with regions around the *E. coli* chromosome, Niki *et al.* (2000) obtained evidence that gene regions tend to cluster near the origin and the terminus of replication. They suggested a circular arrangement of the

replicating nucleoid: rotation of the chromosomal circle would explain the cellular positions of gene regions relative to cell length. However, they based their interpretation on an incorrect assumption about the relationship between cell age and DNA replication (under the growth conditions used, *E. coli* strain K-12 does not contain a so-called B-period, i.e. a period in which no DNA replication takes place after cell birth; for a review, see Helmstetter, 1996).

For a correct interpretation of the movement of subnucleoid DNA regions, a thorough understanding of the relationship between cell growth and DNA replication is essential. This relationship can be determined experimentally (Bipatnath *et al.*, 1998; Huls *et al.*, 1999). To use it, however, growth conditions should be constant, i.e. the culture should be in steady state (Campbell, 1957; Maaloe and Kjeldgaard, 1966; Fishov *et al.*, 1995; Huls *et al.*, 1999).

We wished to determine the position of two subnucleoid DNA regions in between origin and terminus of DNA replication during the cell cycle. One probe recognises the *ftsQ*, *ftsA* and *ftsZ* genes in the 2 min region or *dcw* cluster, where most of the cell division genes are encoded. The other probe is specific for the *minB* region at 25.6 min, which contains the genes *minC*, *minD* and *minE* (De Boer *et al.*, 1988). As a positional reference, we used FISH-labelled *oriC* (cf. Roos *et al.*, 1999).

We found that, on average, the *oriC*, *ftsQAZ* and *minB* regions segregated in the order in which they became replicated. Their average positions followed the separation of the nucleoids. In newborn cells, the duplicated origins were already symmetrically positioned in the cell, whereas the non-replicated *minB* region was asymmetrically located. The *minB* region moved from an off-centre position to a central position upon duplication. The data fitted a model in which DNA replication takes place in the cell centre and gene regions move apart after duplication in line with nucleoid separation (Dingman, 1974; Lemon and Grossman, 1998; Koppes *et al.*, 1999).

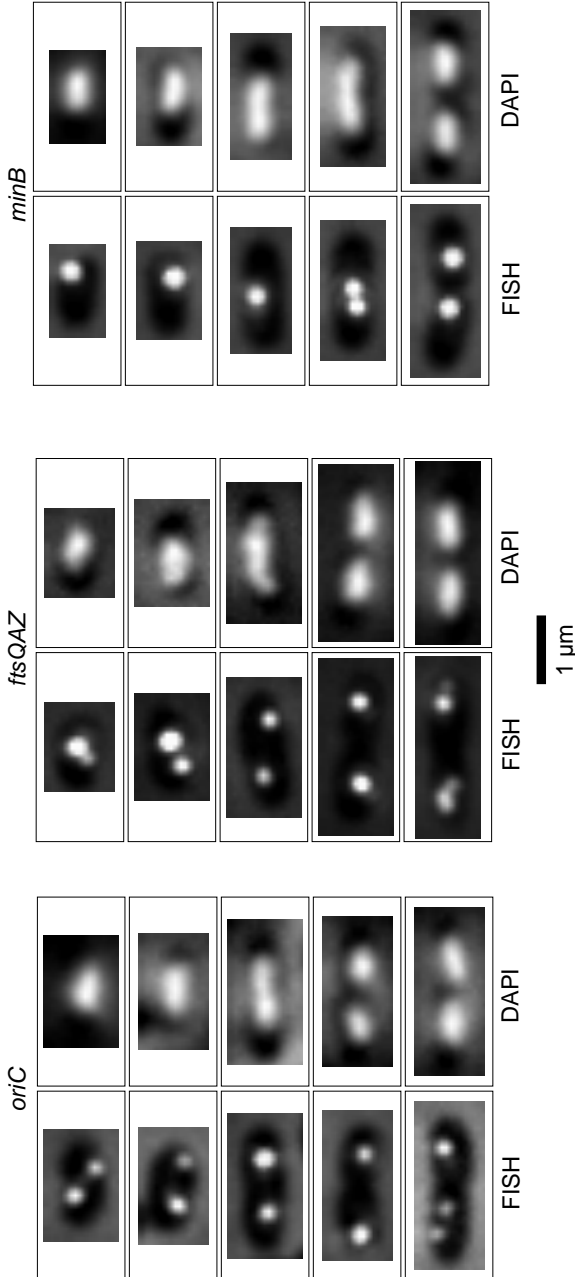


Figure 3.1 - hybridisation foci of *oriC*, *ftsQAZ* and *minB* and DAPI-stained nucleoids in fixed cells of increasing length

Cells and foci were photographed in phase contrast and with an Alexa filter (left); cells and nucleoids were photographed in phase contrast and with a DAPI filter (right). Cells hybridised with *oriC* probe (top); cells hybridised with *ftsQAZ* probe (middle); cells hybridised with *minB* probe (bottom). The bar represents 1 µm.

3.3 Results

The duration of major cell cycle events such as DNA replication and cell division are only reproducible if cultures are grown under steady-state conditions (Campbell, 1957; Maaløe and Kjeldgaard, 1966; Fishov *et al.*, 1995; Huls *et al.*, 1999). The steady-state culture of *E. coli* strain MC4100 that we grew had a doubling time of 79 min, and the periods C (DNA replication time) and D (time between termination of DNA replication and cell division; Helmstetter, 1996) were approximately 70 min and 43 min respectively (Huls *et al.*, 1999; Vischer *et al.*, 1999). By subtracting the sum of C and D from the doubling time, it was derived that DNA replication initiates about 34 min before cell birth (fig. 1.7, top). Thus, in the strain used, the C period is distributed over two subsequent cell cycles. Because cell length can be used as a measure of time in a steady-state culture, we could calculate the expected number of *oriC*, *ftsQAZ* and *minB* DNA regions per cell length from the determined C and D periods (Nanninga *et al.*, 1982 and references therein). Assuming that replication proceeds at a constant rate, we expected two *oriC* regions, two *ftsQAZ* regions and one *minB* region in newborn cells; these numbers double before the next cell division. The cellular positions of *oriC*, *ftsQAZ* and *minB* regions were compared by three DNA hybridisations *in situ* on cells from the same steady-state culture.

3.3.1 Efficiency of FISH labelling

Micrographs of fluorescent foci in various length classes are shown in figure 3.1. In the case of *oriC* and *ftsQAZ*, the images resemble each other and they suggest two foci in small cells. These foci duplicate at longer cell length, whereas in the case of *minB*, one focus is present in small cells, which becomes duplicated towards cell division. Not all cells were labelled or contained the number of expected labelled targets. Only 55% of the cells were labelled in the case of *oriC*, whereas this was 94% for *ftsQAZ*. This indicates that the *ftsQAZ* probe is more effective in labelling its target than the *oriC* probe. To assess this point further, we have listed the expected number of targets per cell for the three gene regions in Table 1. Out of 496 cells successfully hybridised with *oriC* probe, we

observed four foci in only four out of the predicted 291 cells with four targets. We observed two foci in 71 cells out of the remaining 205 cells with two targets. Out of 498 cells successfully hybridised with *ftsQAZ* probe, we observed four foci in one out of 19 cells with four targets, two foci in 183 out of the predicted 465 cells with two targets and one focus in six out of 14 cells with one target.

Table 1. Predicted and observed number of cells from a sample of cells with foci, with calculated number of DNA targets and corresponding number of foci.

Targets per cell	<i>oriC</i>		<i>ftsQAZ</i>			<i>minB</i>	
	2	4	1	2	4	1	2
Predicted	205	289	14	461	19	161	826
Observed	71	4	6	183	1	121	214
Percentage of predicted	35	1	43	40	5	75	26
Percentage or predicted with fewer foci	61	99	NA	42	95	NA	72
Percentage of predicted with more foci	4	0	57	19	0	25	2

Out of 999 cells successfully hybridised with the *minB* probe, we observed two foci in 214 out of the predicted 835 cells with two targets. We observed one focus in 121 out of 164 cells with one target. Clearly, the *minB* probe also did not label all potential targets.

There are a number of possible reasons why the observed number of foci cell⁻¹ is reduced. Foci might be too close together to be resolved by microscopy, foci might be on top of each other and/or targets may not be labelled because DNA regions are not accessible to DNA probes (cf. Roos *et al.*, 1999). These considerations, in our view, underpin the importance of establishing the DNA replication cycle at a particular growth rate to calculate the number of potential FISH targets.

3.3.2 Average position of *oriC*, *ftsQAZ* and *minB* DNA foci

To investigate the intracellular distribution of fluorescent foci with respect to cell length, we measured the distance from the centre of each focus to mid-cell and plotted this against cell length (fig. 3.2A). The scatter patterns of *oriC* foci and *ftsQAZ* foci suggest gradual separation of

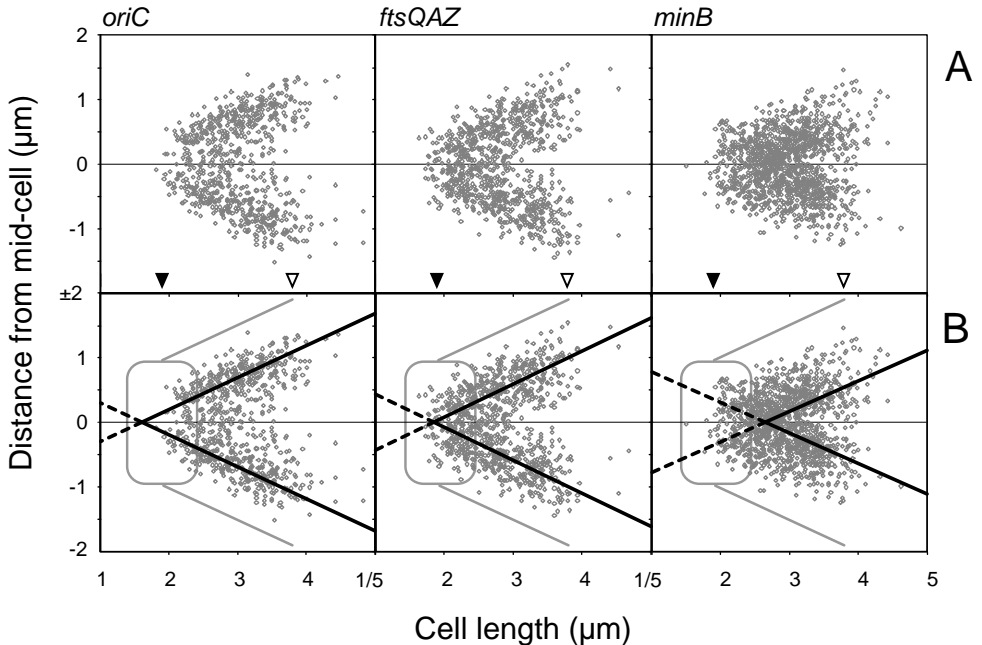


Figure 3.2 - Distances of foci from mid-cell in relation to cell length

Black arrowheads indicate the estimated average size of newborn cells (1.9 µm); open arrowheads indicate the estimated average size of dividing cells (3.8 µm). Negative values refer to the arbitrary left side of a cell; positive values refer to the arbitrary right side of a cell. Black lines represent regression lines (B). Regression coefficients were found using a maximum likelihood procedure (see Appendix). Grey lines represent the cell poles (a newborn cell is drawn to scale in each graph). Regression coefficients: *oriC*, slope = 0.50 ± 0.02 µm, y-intercept = 0.79 ± 0.09 µm; *ftsQAZ*, slope = 0.51 ± 0.11 µm, y-intercept = 0.96 ± 0.34 µm; *minB*, slope = 0.46 ± 0.09 µm, y-intercept = 1.20 ± 0.29 µm.

these regions as cell length increases. The scatter pattern of *minB* foci is more compact, but still suggests gradual separation in longer cells.

Because gradual separation seemed appropriate at a first approximation, we assumed a linear relationship between focus-mid-cell distance and cell length, which enabled us to use a linear regression procedure to estimate the average cellular position as a function of cell length (fig. 3.2B).

Furthermore, we used our predictions about the number of DNA targets (Table 1), assuming that the reduced number of foci was a result of missing label instead of targets clustering together. Note that the slope of the regression line of the distance between a cell pole and mid-cell as a function of cell length is 0.5. The slopes of regression lines through scatter points of *oriC*, *ftsZAZ* as well as *minB* (fig. 3.2B) were not significantly different from 0.5 ($P > 0.05$), suggesting that, on average, separation of DNA regions took place in line with cell elongation. The point where regression lines cross, corresponding to the x-intercept, was denoted as the 'separation point', the point at which duplicated DNA regions start to separate. The separation point of *oriC* was at a cell length of about 1.6 μm (the length of a prospective daughter cell in the previous cycle), which indicates that separation occurred before cell birth (the estimated length of a newborn cell was 1.9 μm). The separation point of *ftsZAZ* foci was at a cell length of about 1.9 μm , and the separation point of *minB* was at a cell length of about 2.6 μm .

As can be seen in figure 3.1, a single *minB* focus is located asymmetrically in the smallest cell, whereas in longer cells, the focus occurs in the cell centre. To assess this further, we made histograms of the *minB* positions in various length classes (fig. 3.3). In the shortest length classes, the average position of a *minB* focus is off centre. Near the *minB* separation point (length class 2.7 μm), the average positions of the two foci are closely apposed, and they separate further when cells become longer (fig. 3.3).

Thus, on average, *oriC* regions separated first, followed by *ftsZAZ* regions and *minB* regions. The asymmetrically located *minB* gene region seems to migrate to the cell centre before duplication.

3.4 Discussion

3.4.1 The interpretation of FISH data

We have analysed the location of subnucleoid DNA regions in *E. coli* relative to cell cycle events, such as cell birth and initiation of DNA

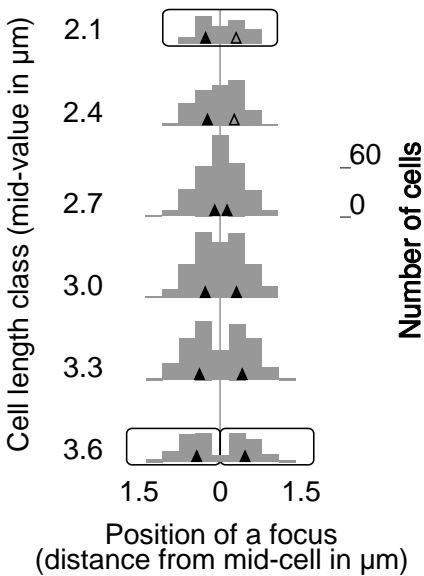


Figure 3.3 - Histograms of the cellular position of minB foci in various cell length classes measured as distance from mid-cell

Foci were randomly placed on the left side of a cell or on the right side of a cell. Arrowheads indicate the average position of foci in a cell length class. Because of randomisation, two average positions are found in cells with one predicted DNA target (cells shorter than 2.4 μm); the second position is indicated by an open arrowhead.

replication. We concluded that, on average, replicated DNA regions segregate in line with cell growth and nucleoid segregation. However, examination of the number of foci found in cells did not directly support this conclusion. The number of predicted DNA targets exceeded the number of visible foci in most cells. For instance, although we expected two origin regions in short cells, many cells contained one focus. An explanation may be that two (or more) DNA regions are too close to be resolved by light microscopy, which would suggest that DNA regions stick together for some time after duplication. Although we do not exclude this possibility, we favour the explanation that not all DNA targets became labelled to produce a visible signal. This is illustrated by the high number of cells with foci of the *fts*QAZ probe (94%) and the low number of cells with foci of the *oriC* probe (55%). The higher efficiency of the *fts*QAZ probe shows that probe DNA can penetrate cells; we have no reason to believe that this is not also true for the *oriC* probe. Our conclusion is that the *oriC* probe did penetrate the cells but, in many cases, did not label its target. For our interpretation of FISH data, we

therefore used the expected number of DNA targets per cell length and not the number of visible foci.

In contrast, the assumption that each DNA target is labelled may lead to a different interpretation of FISH data (cf. Niki and Hiraga, 1998). Under growth conditions ($T_g = 55$ min at 37 °C in M9 medium supplemented with glucose, proline and thiamine) that would also produce two origins at birth (Hiraga *et al.*, 1998; our unpublished results), Niki and Hiraga (1998) detected short cells with one focus and short cells with two well-separated foci. Although the labelling patterns correspond to our findings, they concluded that one of the newly duplicated origins migrates quickly to the other side of the cell. However, we believe that, for the interpretation of FISH data, the efficiency of the technique should be taken into account. If a single focus is present, one has to choose between the possibility that a duplicated gene region cannot be resolved or whether a focus is lacking because a target has not been labelled. According to these considerations, we interpret our *oriC* data to mean that, in a newborn cell, when 50% of the genome has been replicated, the two *oriC* targets are on average well separated (this paper and cf. Roos *et al.*, 1999).

3.4.2 Separation of DNA regions

We have looked at the separation behaviour of two regions at different locations on the *E. coli* chromosome [*ftsZAZ* (2 min) and *minB* (26.5 min)] using the position of *oriC* as a reference (fig. 3.2). As for *oriC*, our data suggest that, on average, these regions move apart in line with cell elongation as soon as they are detectable after duplication and that separation follows the order of their replication. Thus, the average position of *ftsZAZ* was in between the origin and *minB*. In *Bacillus subtilis*, the cellular positions of two opposite gene regions in between origin and terminus have been compared (Teleman *et al.*, 1998). It was found that these regions not only occupied comparable positions in the cell, they also occurred in between origin and terminus. Our *E. coli* data therefore closely resemble those obtained with *B. subtilis*.

Note, however, that the above analysis refers to average positions of the foci and also note that considerable scatter occurs around the separation points (fig. 3.2). This makes it difficult to assess whether initial separation after duplication of a gene region occurs in line with cell elongation. In individual living cells of *E. coli* and *B. subtilis*, DNA regions close to the origin have been shown to separate non-gradually by time-lapse microscopy (Gordon *et al.*, 1997; Webb *et al.*, 1998 respectively). We can reconcile these results with our data, if we assume that movement of DNA regions is the sum of an overall movement along with nucleoid segregation and of local short-distance movements unrelated to nucleoid movement. In our analysis, local short-distance movements would be averaged out. They would instead constitute an important part of the observed variation in distances of foci from mid-cell. This implies that the expected result of our method would be gradual movement, whereas this would not necessarily be so in the case of an individual cell.

3.4.3 DNA segregation and the replisome

Recent evidence in *B. subtilis* (Lemon and Grossman, 1998) and in *E. coli* (Koppes *et al.*, 1999) indicates that DNA replication occurs in the cell centre. These observations are compatible with the replisome model of Dingman (1974), in which bi-directional DNA replication takes place at a fixed cellular site (the replisome) and the DNA strands to be replicated move through the replisome. In this model, duplicated origins move apart during DNA replication. How do our data fit into this model? In a newborn cell, the two origins have already separated, because they were duplicated in the previous cell cycle (fig. 1.7, top). In contrast, the *minB* region has not yet duplicated, and it is located asymmetrically in the cell near the youngest pole (fig. 3.1). Presumably, this asymmetry has arisen because the *minB* regions were closer to mid-cell during the preceding division. When the newborn cells become longer, the *minB* region goes to mid-cell towards the replisome, whereas the origins move further apart while maintaining a constant distance to the respective nearest poles (Roos *et al.*, 1999). Obviously, *minB* and one of the *oriC*s

have to pass each other. How can these opposing DNA movements be visualised? The original Dingman figure with the terminus perpendicular to the fixed replisome has been redrawn in figure 3.4A. Bending of the terminus towards mid-cell brings *minB* into an asymmetrical position on the length axis of the cell (fig. 3.3 and fig. 3.4B). During DNA replication, *minB* approaches the replisome at mid-cell (fig. 3.3) and passes one of the replicated *oriC*s that moves in the opposite direction (fig. 3.4C). Thus, two different movements are responsible for the passing of *oriC* and *minB* along each other. On the one hand, duplicated origins separate, initially independently and later on in line with cell elongation. On the other hand, the asymmetrically located *minB* region migrates to the cell centre, possibly pulled by the active replisome.

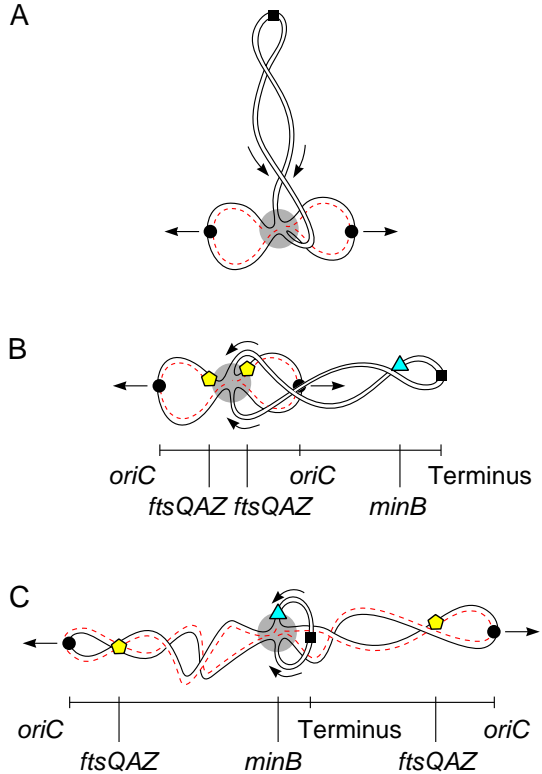
It has been suggested (Niki *et al.*, 2000) that, in order to start replication, *oriC* would move from a polar position to mid-cell, and this rearrangement has been supposed to take place during the *B* period. The *B* period is, by definition, the time between cell birth and initiation of DNA replication, provided that the *C* period is contained within the same cell cycle, i.e. when $C + D < T_d$ (Helmstetter, 1996). This has been observed in *E. coli* B/r strains, in particular at slow growth rates ($T_d > 60$ min; for instance, see Koppes *et al.*, 1999). So far, a *B* period has not been observed in K-12 strains with T_d s of 19230 min (Helmstetter, 1996). In our view, these considerations argue against the existence of a *B* period in *E. coli* K-12 strains for the repositioning and activation of *oriC*.

3.4.4 Concluding remarks

We emphasise that our data refer to average movement of DNA regions during the cell cycle. Many of the forces behind the presumed movements of DNA regions have not been found yet. A mitotic-like mechanism that actively segregates DNA has been suggested (for a review, see Glaser *et al.*, 1997; Shapiro and Losick, 1997; Sharpe *et al.*, 1998), and it has also been suggested that this mechanism exists alongside a mechanism for gradual movement of bulk DNA (Sharpe *et al.*, 1998). We would like to add the possibility that DNA regions exhibit

Figure 3.4 - Model for sequential separation of DNA subregions on the *E. coli* chromosome

A. Redrawing of the original Dingman figure with the terminus perpendicular to the fixed replisome, shortly after initiation of DNA replication (Dingman, 1974).
 B. Adaptation of (A) with the terminus-containing loop in the length axis of the cell. C. Organisation of DNA subregions before termination of DNA replication. Arrows indicate the direction of movement of DNA (towards the replisome or away from the replisome in the length axis of the cell). Black circles, *oriC* region; black squares, terminus region; yellow pentagons, *ftsQAZ* region; blue triangles, *minB* region; red dashed line, newly synthesised DNA. Note that, as replication progresses from (B) to (C), *minB*, moving towards the replisome, passes one of the *oriCs*, which is moving away from the replisome.



constrained diffusional motion, similar to that found in yeast and *Drosophila* (Marshall *et al.*, 1997).

3.5 Experimental procedures

3.5.1 Bacterial strains and growth conditions

Cells of *E. coli* strain LMC500 [MC4100 (*F*, *araD139*, (*argF-lac*) U169, *deoC1*, *flbB5301*, *ptsF25*, *rbsR* *relA1*, *rpsL150*) *lysA*] (Taschner *et al.*, 1988) were grown at 28 °C in steady state with a doubling time (T_d) of 79 min in glucose minimal medium containing 6.33 g/l $K_2HPO_4 \cdot 3H_2O$, 2.95 g/l KH_2PO_4 , 1.05 g/l $(NH_4)_2SO_4$, 0.10 g/l $MgSO_4 \cdot 7H_2O$,

0.10g/1 MgSO₄·7H₂O, 0.28mg/1 FeSO₄·7H₂O, 7.1mg/1 Ca(NO₃)₂·4H₂O, 4mg/1 thiamine, 4g/1 glucose and 50mg/1 lysine (pH7.0). We harvested 50ml of cell culture at an OD₄₅₀ of 0.2 and fixed cells in 1ml of 1% OsO₄ in TY medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 3 mM NaOH, pH7.0), which was stored at 4 °C.

3.5.2 Probes

We used an *oriC* probe obtained from plasmid pOC162 (kindly provided by Dr W. Messer, Max Planck Institut für Molekulare Genetik, Berlin, Germany). This plasmid contains the 248bp *oriC* region with 3kbp on each side of *oriC*. Because pOC162 contains no sequence homologies to chromosomal sequences, the whole construct was labelled and used as a probe for FISH. The *ftsZAZ* probe was obtained from plasmid pZAZ, which contains the *ftsZAZ* operon (kindly provided by R. d'Ari, Institut Jacques Monod, CNRS, Université de Paris, France). To obtain a probe without any other chromosomal sequences from the plasmid, a 4.4kbp *EcoRI*/*NotI* fragment containing *ftsZAZ* was excised and labelled. We obtained the *minB* probe from plasmid pDB103, a plasmid that contains the complete *minB* operon of *E. coli* (De Boer *et al.*, 1988). To obtain our probe without other chromosomal sequences from the plasmid, a 1kbp segment containing part of the *minD* gene and part of the *minE* gene was amplified by polymerase chain reaction (PCR) and labelled.

3.5.3 Probe labelling

Plasmids were labelled with digoxigenin-11-dUTP (DIG; Hoffmann-La Roche) by nick translation. DIG incorporation was checked by spot blotting on Hybond nylon filter (Hoffmann-La Roche) and detection with mouse anti-DIG alkaline phosphatase-conjugated antibodies (Hoffmann-La Roche) and 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT; Hoffmann-La Roche) as substrate. The specificity of the probe was checked by Southern hybridisation on digested genomic DNA of strain MC4100 (Sambrook *et al.*, 1989).

3.5.4 Preparation of cells for fluorescence microscopy

Cells were washed in PBS (140 mM NaCl, 27 mM KCl, 10 mM Na₂HP₄ and 2 mM KH₂P₄, pH7.2) and post-fixed in 0.5% formaldehyde and 0.04% glutaraldehyde in TBS (10 mM Tris and 0.9% NaCl, pH7.5) for 15 min at room temperature. Fixed cells were centrifuged at 3000g for 5 min, washed three times in PBS and subsequently incubated in 0.1% Triton X-100 in PBS for 45 min at room temperature. Cells were washed three times in PBS and incubated in PBS containing 100µg/ml lysozyme (Sigma-Aldrich) and 5 mM EDTA for 45 min at 37 °C. After washing three times in PBS, the cells were incubated with 100µg/ml RNase A (Hoffmann-La Roche) in PBS for 60 min at 37 °C. After washing three times in PBS, 10µl of cell suspension was applied to slides coated with 0.01% poly L-lysine, covered with a coverslip (15 mm diameter) and left for 20 min at room temperature. Then, the slides were washed in a Coplin jar with 2× SSC (sodium salt citrate; 0.15M NaCl, 150 mM sodium citrate, pH7) and put on top of coverslips with 10µl of probe solution [1ng/µ probe in hybridisation mix, containing 2×SSC with 300 µg/ml herring testes DNA, 5% polystyrenesodium sulphate 100000 (Acos), 1× Denhardt's reagent (Sambrook *et al.*, 1989) and 50% formamide]. The preparations were denatured for 5 min at 80 °C. Hybridisation occurred overnight in a moist chamber at 37 °C. Unbound probe was washed three times for 10 min at 40 °C in a Coplin jar with 1× SSC and 50% formamide (pH7). Slides were subsequently washed in 2×SSC and TN buffer (100 mM Tris-HCl, pH7.5, 150 mM NaCl).

Before immunofluorescence staining, non-specific binding sites were blocked by incubating the cells in TNB [0.5% (w/v) blocking reagents (Hoffmann-La Roche) in TN] for 30 min at 37 °C. DIG-labelled probe detection was carried out with mouse anti-DIG (Hoffmann-La Roche), 1:500 in TNB, rabbit anti-mouse-conjugated Cy3 (Jackson ImmunoResearch Laboratories), 1:400 in TNB and goat anti-rabbit-conjugated Alexa 546 (Molecular Probes), 1:600 in TNB. Slides with antibodies were incubated in a moist chamber at 37 °C for 60 min. Between incubations with antibodies, the slides were washed three times

for 5 min in TNT [TN with 0.05% (v/v) Tween 20]. After immunodetection, slides were rinsed three times in TNT, once in TN and once in PBS. The preparations were mounted in 5µl of PBS with DAPI (500ng/ml 2,4,6-diamidino-phenylindole).

3.5.5 Microscopy and image analysis

Preparations were photographed with a cooled Princeton CCD camera mounted on an Olympus fluorescence microscope (BH2-RFC) equipped with a 100W mercury lamp. Images were made using the program IPLAB spectrum 3.0 (Signal Analytics). Cells and fluorescent foci were photographed using a combination of phase contrast and a filter combination for Alexa 546 (illumination 510-550nm; emission 590nm). To check nucleoid morphology, cells and DAPI-stained DNA were photographed using a combination of phase contrast and a filter combination for DAPI (illumination 300-400nm; emission 420nm). Length, position of foci and nucleoid length of each cell were determined interactively. Interactive measurements were performed as 'structured point collection' on an Apple computer (Power Macintosh 7100) using the public domain program OBJECTIMAGE 1.62n (Vischer *et al.*, 1994; <http://simon.bio.uva.nl/object-image.html>), which is based on the NIH IMAGE software (W. Rasband; <http://rsp.info.nih.gov/nih-image>).

3.6 Acknowledgements

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3.7 References

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3.8 Appendix

Regression lines were calculated using a linear regression model:

$$d(l) = A + B \cdot l$$

in which $d(l)$ is the distance from mid-cell at cell length l , A is the y -intercept of the regression line, and B is the slope of the regression line.

Regression coefficients A and B were found using a maximum likelihood procedure. We assumed that distances were distributed normally around a mean distance at each cell length, and we took into account that foci can be positioned on the left side of a cell as well as on the right side of a

cell (negative and positive distance values respectively). We used the following likelihood function:

$$L_{l,s}(m\delta) = [N_{-\delta(l),s}(m\delta) + N_{+\delta(l),s}(m\delta)] / 2$$

in which $L_{l,s}$ represents the likelihood that measured distance $m\delta$ is found at cell length l , and N is a Gaussian likelihood function with mean $\delta(l)$ or $-\delta(l)$ and standard deviation s . For s , we assumed a value of $0.2 \mu\text{m}$. We used a bootstrap method to find those A and B for which the sum of likelihoods of all measured distances was maximal.