

EXPERIMENTS ON MOVEMENT OF DNA REGIONS IN *ESCHERICHIA COLI* EVALUATED BY COMPUTER SIMULATION

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4.1 Summary

During the cell cycle of *Escherichia coli*, DNA is replicated and segregated over two prospective daughter cells. Nucleoids as a whole segregate gradually, in line with cell elongation, but sub-nucleoid DNA regions may behave differently, segregating non-gradually. We tested the ability of three models to predict the outcome of a fluorescent *in situ* hybridisation (FISH) experiment. We did this by comparing computer-simulated data with experimental data. The first model predicts gradual segregation in line with cell elongation. The second model predicts that origins stick together for some time after duplication before one copy jumps to the other side of the cell (non-gradual segregation). The simulated data of these models were very similar, indicating that FISH is not a suitable

method to distinguish between these two models. The third model predicts that origins may be anywhere within the nucleoid(s). We found that simulated data using the third model resemble the experimental data most. However, DNA regions are not randomly localised in the cell, although their localisation is fuzzy. We propose that movement of DNA regions is the result of a combination of factors. Nucleoid segregation (or the forces behind it) dictates the overall direction of movement. Other factors, of which we show that diffusion could be an important one, move DNA in other directions giving rise to non-gradual movement in individual cells and contributing to variation in intracellular position per cell length in a population of cells.

4.2 Introduction

Cell growth, transcription, replication and DNA segregation occur simultaneously and with high efficiency in bacteria. It is likely that the (dynamic) organisation of DNA in the cell is essential to reach this efficiency. Therefore, movement of replicated DNA to the prospective daughter cells is probably the result of one or more mechanisms (for a recent review see Gordon and Wright, 2000). Nucleoids as a whole have been shown to segregate gradually in line with cell elongation in *E. coli* (van Helvoort and Woldringh, 1994) and in *Bacillus subtilis* (Sharpe *et al.*, 1998), but how do individual DNA regions move?

Our studies on fixed cells in which DNA regions were fluorescently labelled *in situ* via hybridisation with a DNA probe (FISH), suggested that, on average, sub-nucleoid DNA regions move gradually in line with nucleoid segregation (Roos *et al.*, 1999; 2001). However, the FISH data showed a lot of variation in intracellular position when plotted as a function of cell length; a typical example is the origin of replication of *E. coli*, *oriC* (fig. 4.1). Additionally, movement of sister DNA regions directly after duplication could not be addressed by FISH, because DNA regions are too close to each other to be resolved by light microscopy. Thus, statistical treatment of FISH data can only give information about average movement of DNA regions as a function of cell length of a

(steady-state) population (Roos *et al.*, 2001).

Movement of DNA regions in individual living cells can be studied by tagging DNA regions by green fluorescent protein (GFP; see Margolin, 2000 and references therein). GFP tagged regions close to *oriC* or containing *oriC* have been shown to segregate much quicker than cell elongation in *E. coli* (Gordon *et al.*, 1997) and in *B. subtilis* (Sharpe and Errington, 1998; Webb *et al.*, 1998). This supports models which predict that duplicated DNA regions segregate quickly, not in line with cell elongation, and possibly in an active, mitotic-like process (see for instance Sharpe and Errington, 1999 and references therein). Consequently, Niki and Hiraga explained their FISH data with a model in which one copy of *oriC* 'jumps' to the other side of the cell after duplication (Niki and Hiraga, 1998).

Even if nucleoids segregate in a different way than sub-nucleoid DNA regions, a model of movement of DNA regions in bacteria as function of cell length should explain average movement of DNA in a (steady-state) population of cells as well as movement of DNA in individual living cells. However, it can be difficult to see if a particular model explains the observed data sufficiently. An objective method to test the validity of a model is to compare computer simulated data based on such a model with experimental data for a population of cells. We have done this using three models of DNA movement in *E. coli*. Model I predicts gradual segregation of DNA regions in line with cell elongation; model II predicts that DNA regions segregate non-gradually after replication; model III predicts that DNA regions have no preferred position within the nucleoid. We present here the results for the origin of replication in *E. coli*, *oriC*, because this is probably the best studied DNA region. Our results indicate that DNA organisation in *E. coli* is not random, although it is fuzzy, i.e. labelled DNA regions are not precisely located in the cell during the cell cycle. We discuss a model in which DNA movement is the result of a number of factors. Nucleoid segregation, or the force(s) behind it, is one of these factors and 'diffusion in a confined region' (cf. Marshall *et al.*, 1997) is one additional factor.

4.3 Materials and methods

4.3.1 Simulation of FISH data

For each cell from a population, theoretical positions were calculated for each copy of *oriC* present in the cell according to three models (see appendix, section 4.3). Because no distinction could be made between the two sides of a cell, we randomised cell orientation by multiplying every distance in a cell by +1 or -1 (numbers obtained from Microsoft Excel's Bernoulli random number generator with $p = 0.5$). To each theoretical position a random value drawn from a normal distribution with mean 0 and a standard deviation of $0.2 \mu\text{m}$ was added. This value for the standard deviation was derived from previously published data (Roos *et al.*, 2001). FISH labelling efficiency was incorporated by randomly selecting DNA regions in the data set with an experimentally derived probability $p = 0.3$ (see appendix, section 4.3). If DNA regions were closer to each other than the experimentally derived value of 250 nm (see below), then they were scored as one focus with its centre in between the DNA regions. Finally, a measure of error was incorporated by adding a random value drawn from a normal distribution with mean 0 and a standard deviation of 15 nm (see below).

4.3.2 Measurement error

To determine the measurement error of manually indicating the centre of a FISH focus in an image, we created a thousand images with a simulated FISH focus at various positions. An experimenter scored the centre of the focus in each image manually. The standard deviation of the average difference between the positions of the simulated FISH foci and the positions indicated by the experimenter was used as measurement error ($0.015 \mu\text{m}$). FISH foci were simulated by a 2-D normal intensity distribution with a standard deviation similar to that of a FISH focus (approximately $0.14 \mu\text{m}$).

4.3.3 Resolution of fluorescence microscopy

We estimated the resolution of fluorescence microscopy by scoring if two foci could be resolved visually in a thousand images with two simulated FISH foci at various distances from each other. The average distance at which two foci could be resolved was used as a measure of resolution (250 nm).

4.3.4 Diffusion in a confined region

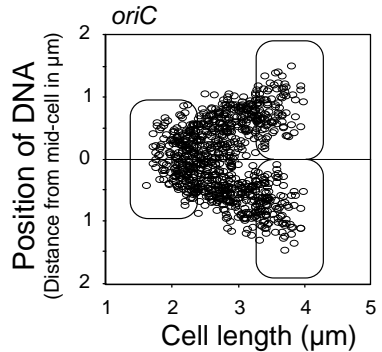
To simulate positions that are the result of diffusion in a confined region we calculated the position that a putative DNA region reaches after a random walk of 10^6 equidistant steps of 0.075 μm in random directions within a sphere with a radius of 0.3 μm (cf. Marshall *et al.*, 1997), and we added that position to the intracellular position of the confined regions as determined by the theoretical positions of model I and model II, respectively.

4.4 Results

In order to evaluate models of movement of *oriC* in slow growing *E. coli* K-12 cells, we first calculated the theoretical position of *oriC* as a function of cell length according to each of the three models (fig. 4.2A). Model I predicts that segregation of origins proceeds at exactly the same speed as cell elongation. Consequently, origins remain at a fixed distance from the nearest (prospective) cell pole. Model II is equal to model I except that after duplication the two duplicated origins first stick together, jointly moving away gradually from the cell centre on one side of the cell. When half of the cell length between initiation of DNA replication and termination of DNA replication is reached, one of the origins 'jumps' to the other side of the (prospective daughter) cell. In model III DNA has no preferred position: a DNA region has an equal probability to be anywhere within the nucleoid. For each model, basic assumptions about cell size, growth rate, and replication rate were chosen conform our previously published data (Huls *et al.*, 1999; Roos *et al.*, 2001). Thus, because replication initiated in a previous cell cycle, there are two copies

of *oriC* at cell birth in all models. Except for model III, the position of the origins at initiation is at $\frac{1}{4}$ and $\frac{3}{4}$ of the cell, because we assumed that replication takes place in the centre of each prospective daughter cell when replication initiates before cell birth (cf. Koppes *et al.*, 1999; Lemon and Grossman, 1998).

Figure 4.1 - Distances of *oriC* foci from mid-cell as a function of cell length. Reprinted from figure 3.2.



We simulated FISH experiments by calculating the theoretical intracellular positions of *oriC* for a population of cells and incorporating the following factors: measurement error, resolution of fluorescence microscopy, the probability that an *oriC* region is labelled and a normally distributed variation in the intracellular position of *oriC* regions (fig. 4.2B). If we compare the simulated data of model I and model II, we find that, although the bases of these models differ, the difference in simulated data is small. If we compare the simulated data of these models with the simulated data of model III, we find that models I and II lack data points in the cell centre when compared to model III. If we compare our simulated data (fig. 4.2B) with the experimental data of figure 4.1, then model I and model II both lack data points in the cell centre. So far, model III seems to resemble the experimental data most. To investigate these results quantitatively we calculated the 'likelihood' of the experimental data of figure 4.1 to be the result of each of the three models (fig. 4.2). In agreement with our visual comparison we found that the experimental data are less likely the result of model I or model II than the result of model III. Note that the data plots and the likelihood-values of model I and model II are very similar, indicating that FISH is not a suitable method for discriminating between these two models.

In the simulations above we assumed a value of $0.2 \mu\text{m}$ for the standard

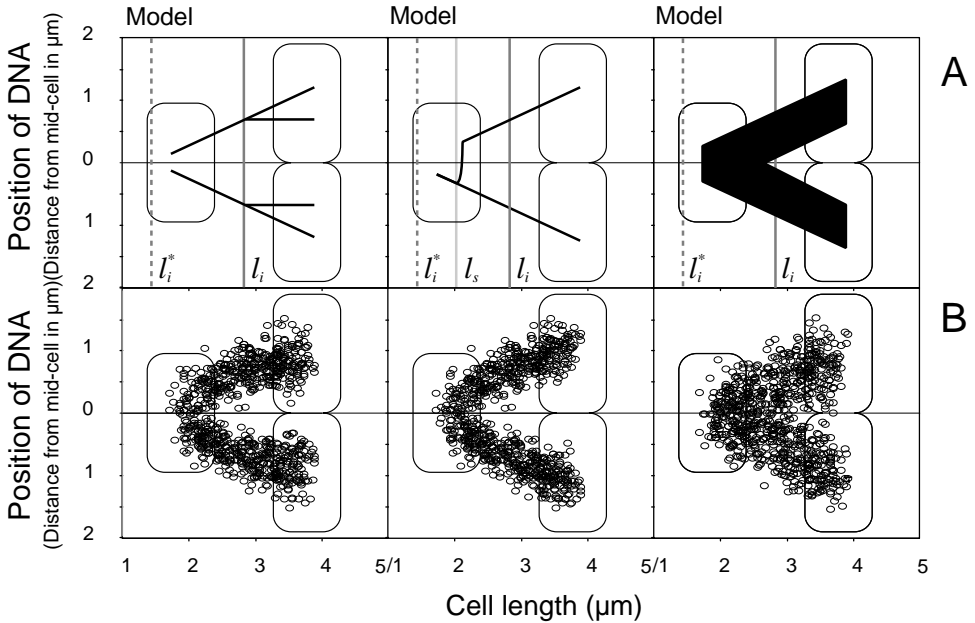


Figure 4.2 - Theoretical (A) and simulated (B) distances of *oriC* regions from mid-cell as a function of cell length as predicted by three different models

Model I predicts gradual separation of duplicated *oriC* regions in line with cell elongation; **Model II** predicts that duplicated *oriC* regions stick together until a cell reaches a cell length halfway between initiation of DNA replication and termination of DNA replication, after which one of the copies ‘jumps’ to the other side of the cell. **Model III** predicts that DNA regions are distributed uniformly over the nucleoid. FISH data was simulated by incorporating measurement error (standard deviation = 15nm), resolution of fluorescent microscopy (250 nm), FISH labelling efficiency ($p = 0.3$), and normally distributed variation in distance between foci and mid-cell (standard deviation = 0.2 μm estimated from experimental FISH data; see Roos *et al.*, 2001). Likelihood values of experimental data of figure 4.1 to be the result of model I, II and III were 321, 324, and 372, respectively. l_i^* , virtual cell length at initiation of DNA replication before cell birth; l_i , cell length at initiation of DNA replication; l_s , cell length when sister copies of *oriC* separate.

deviation of distance from mid-cell, because this was the estimated value of our experimental data (Roos *et al.*, 2001), but what is the source of this variation? The measurement error was 15 nm, which is much smaller than the observed variation. Indeed, a plot of simulated data as function

of cell length using only measurement error as a source of variation looked very similar to the theoretical lines of figure 4.2A (data not shown). Incorporating resolution and FISH labelling efficiency had an effect on the average number of foci cell, but no noticeable effect on the amount of variation. This means that other sources of variation exist.

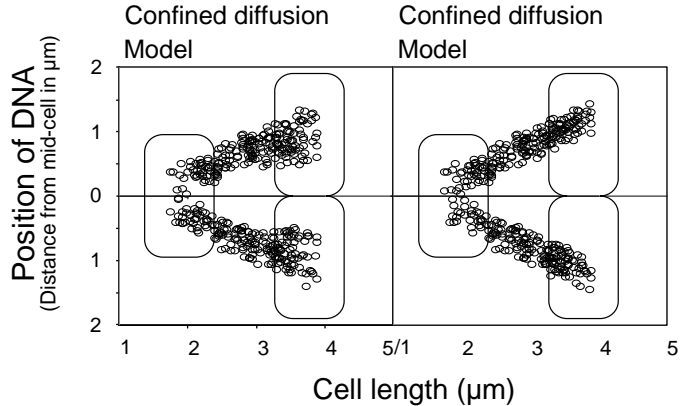
To test if diffusion might be a source of variation, we simulated data using a model in which a DNA region moves by diffusion within a spherical region with a radius of 0.3 μm (cf. Marshall *et al.*, 1997). We produced two data sets using the theoretical positions of model I and model II to define the positions of the confined regions (fig. 4.3). The distributions of simulated data points per cell length of the two confined diffusion models resemble those of model I and model II (compare fig. 4.2B with fig. 4.3), although the distribution of the confined diffusion models are best described by a quadratic function instead of a normal distribution function. These results indicate that diffusion could be an important source of variation. However, diffusion in a spherical confined region with a radius of 0.3 μm did not explain the number of foci found in the cell centre of small to intermediate sized cells. This implies that additional factors are required to explain the distribution of *oriC* regions per cell length.

4.5 Discussion

In this study we show by comparing computer-simulated data with experimental data that DNA regions in *E. coli* may not be precisely localised; of the three models tested the model that predicts random distribution of the origins of replication within the nucleoid, most closely resembled experimental data. However, a 'random model' predicts equal distributions for every DNA region, whereas previous work showed that this is not the case; the average position of two regions in between origin and terminus, *ftsZAZ* and *minB*, analysed by FISH, differed from the average position of the origin (Roos *et al.*, 2001). Moreover, although the positions of regions in a cluster around the origin were similar and the positions of regions in a cluster around the terminus were also similar,

Figure 4.3 - Distance from mid-cell as a function of cell length as predicted by 'diffusion in a confined region'

The theoretical positions of model I and model II (figure 4.2A) were used to determine the central position of the confined regions. The confined region was a sphere with a radius of 0.3 μm .



the positions of regions in between these clusters and the positions of the clusters themselves were different (Niki *et al.*, 2000). This means that the organisation of sub-nucleoid DNA regions in growing *E. coli* may be fuzzy, but it is not random.

The first model that we tested predicts gradual segregation at a speed exactly equal to the speed of cell elongation (fig. 4.2A, model I). Our previous studies showed that the slope of the linear regression lines through the data points of three regions on the *E. coli* chromosome, *oriC*, *ftsZAZ*, and *minB*, did not significantly differ from 0.5, which corresponds to gradual segregation at the speed of cell elongation (Roos *et al.*, 2001). We should stress, however, that we used linear regression to find an estimate of the average position of foci within cells of increasing length. Non-gradual motion of DNA regions during the cell cycle cannot be ruled out. For instance, whether separation is gradual directly after duplication of a DNA region cannot be visualised by methods based on light-microscopy, because of the limited resolution of these methods. However, if initial separation would be gradual, then the cell length at which the regression lines cross should correspond to the cell length at which DNA regions start segregating as calculated from the C and D periods. We calculated cross-points and cell lengths at initiation of replication from previously published data (Roos *et al.*, 2001) and indeed found that these values correspond: for *oriC* 1.6 μm and 1.4 μm

respectively, for *ftsZAZ* 1.9 μm and 1.8 μm respectively, and for *minB* 2.6 μm and 2.4 μm respectively. These properties make the gradual segregation model an attractive model, although the simulated data based on this model did not sufficiently explain the data points observed in the cell centre of small to intermediate length cells (fig. 4.1).

The second model that we tested was based on GFP studies that indicated quick non-gradual movement of duplicated DNA regions after replication (fig. 4.2B; Gordon *et al.*, 1997; Sharpe and Errington, 1998). Non-gradual movement of the origin has also been used to explain FISH data (Niki and Hiraga, 1998), implicitly assuming that in cells with fluorescent signal all DNA regions were labelled. We have argued that this is probably not the case for FISH (Roos *et al.*, 2001). Therefore, we assumed here that inefficient labelling of DNA regions reduces the number of observed foci per cell (model I), or that, in addition, sister origins remain together until halfway between the length at initiation of replication and the length at termination of replication (model II). The 'jump' that follows in model II, should occur after cell birth, because otherwise it would offer no explanation for the occurrence of large numbers of small cells with a single focus as is observed in FISH experiments (Niki and Hiraga, 1998; Roos *et al.*, 2001). This implies that the origin jumps after 50% of the DNA is replicated, because according to our calculations nearly 50% of the *E. coli* chromosome has been replicated at birth (Huls *et al.*, 1999; Roos *et al.*, 2001). In any case, the simulated data of the first and the second model were very similar. In our view, this means that FISH is not a suitable method to distinguish between a gradual model and a model in which DNA regions stick together for some time before quickly segregating. The small difference we found between the likelihood of the experimental data to be the result of these two models supports this conclusion. Consequently, our results neither support nor rule out non-gradual, 'mitotic-like' segregation of DNA regions.

However, we do show here that both the model for gradual segregation and the model for non-gradual segregation are insufficient to explain the FISH data completely.

The 'fuzzy' nature of DNA localisation is reflected by the large amount of variation in experimental data. A standard deviation of 0.2 μm of the average distance of foci from mid-cell is substantial for an organism with a width of about 1 μm and a cell length of a newly born cell of about 1.9 μm . Because FISH is not a gentle method, it could enhance variation. One would expect that different labelling protocols would lead to different amounts of variation. However, FISH data obtained using a protocol slightly different from ours (Niki and Hiraga, 1998), and data obtained using a fundamentally different protocol using GFP (Gordon *et al.*, 1997) show similar amounts of variation to our data. Unfortunately, we could not compare variation quantitatively, because measures of variation were either incompatible or absent. We have tried to quantify some experimental artefacts, such as measurement error, resolution of fluorescent microscopy, and FISH labelling efficiency (the probability that a DNA target is indeed labelled by probe), but the effects on overall variation were marginal (data not shown). Therefore, it seems likely that a substantial amount of variation is caused by other, biologically relevant, factors.

One of the factors that may contribute to variation in DNA localisation is diffusion, because it moves DNA in directions other than those belonging to nucleoid segregation. In yeast and *Drosophila* it has been shown that DNA regions move as a function of diffusion, but that diffusion is limited to a confined region (Marshall *et al.*, 1997). We have tested this idea for *E. coli* by computer simulation and found that diffusion in a confined region could be responsible for a substantial amount of movement of DNA in bacteria (fig. 4.3). Irregular motion of GFP-tagged DNA regions has been reported earlier (Gordon *et al.*, 1997), but to the best of our knowledge, diffusion of individual DNA regions in bacteria has not been addressed directly. Our preliminary results of a time-lapse experiment in *E. coli* with GFP-tagged DNA regions in cells in which all active processes were blocked by NaN_3 (effectively ruling out any movement except diffusion) indicated that DNA regions indeed show substantial diffusional motion, which would allow a DNA region

to cross the width of a cell easily within 20 seconds. Marshall (Marshall *et al.*, 1997) argued that diffusion may be sufficient for the motion required for most of the nuclear processes in eukaryotic nuclei. We suspect that diffusion is at least as important in bacteria, because bacteria are so small.

Based on the analysis described here, we propose that several independent forces determine the position of a DNA region in a growing cell (fig. 4.4). In our view, nucleoid segregation, or the forces behind it, determines the direction of the average movement of DNA regions, which is gradual in line with cell elongation (van Helvoort and Woldringh, 1994). Other factors are responsible for the movement of DNA in various other directions giving rise to non-gradual movements in individual cells, but contributing only to variation when a population is studied. If diffusion is important, it is interesting to see how and to what extent diffusion is constrained: we theoretically tested a spherical confinement region with a radius of 0.3 μm (cf. Marshall *et al.*, 1997), but this was not sufficient to explain the relatively high number of foci found in the centre of small to intermediate sized cells. Overall movement of DNA regions is probably not solely connected to cell elongation; nucleoids in fast growing cells appear triangular in shape, suggesting that origins are not always oriented in the length axis of the cell.

4.6 Acknowledgements

The authors would like to thank Conrad Woldringh for support and discussion.

4.7 References

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4.8 Appendix

4.8.1 Determination of the cell length at initiation and termination of DNA replication

We assume that within the time period $0 \leq t \leq T_d$ cell length growth in a

steady-state population is exponential (Koppes *et al.*, 1973), thus

$$l(t) = 2^{\frac{t}{T_d}} \cdot l_0 \quad (1)$$

in which T_d = the moment of cell division, and l_0 = the length of a new born cell.

Consequently

$$\ln \{l(t)\} = \frac{\ln 2}{T_d} t + \ln(l_0) \quad (1)$$

The moment of initiation of DNA replication, t_i , is derived from

$$t_i = T_d - (C + D)$$

in which C = duration of one round of replication, D = period from termination of replication to cell division. Consequently, the length at the moment of initiation of DNA replication, l_i^* , is found by:

$$l_i^* = l(t_i) = 2^{\frac{t_i}{T_d}} l_0$$

or

$$l_i^* = 2^{\frac{T_d - (C + D)}{T_d}} l_0$$

Similarly the length at termination (or end) of DNA replication, l_e , was derived by substituting t in (1) by the moment of termination of DNA replication

$$t_e = T_d - D$$

Experimental values of the model-parameters are $T_d = 79$ min., $C = 70$ min., and $D = 42$ min. (Huls *et al.*, 1999). So $t_i = -33$ min.,

$l_i^* = 0.75 l_0$, $t_e = 37$ min, and $l_e = 1.4 l_0$. Thus replication initiates before cell birth in a virtual cell with length l , corresponding to a prospective daughter cell before cell division (Roos *et al.*, 2001).

4.3.2 Modelling the distance of *oriC* from mid-cell

The theoretical position of the origin of replication of *E. coli*, *oriC*, was represented as the distance of that DNA region from mid-cell as a function of cell length. Definitions:

- $d = d_r^n$ = distance from cell centre of copy number r out of n copies of *oriC* present in the cell
- l = cell length
- l_0 = length at birth of the cell
- l_i = virtual length at initiation of DNA replication before cell birth
- l_s = length when sister copies of *oriC* separate
- l_i = length at initiation of DNA replication
- l_e = length at termination of DNA replication
- l_d = length of a dividing cell
- L = length of a nucleoid

4.3.2.1 Model I (fig. 4.4A)

For model I we assume that each of the two or four copies of *oriC* in the cell increases its distance from mid-cell (or from the centre of a prospective daughter cell) according to the model

$$d = b \cdot l + a \tag{2}$$

in which the slope $b = 1/2$ by definition, because in a steady-state population the distance between a DNA region and the centre of a prospective daughter cell at cell division is equal to the distance between a DNA region and the cell centre at cell birth. By assuming that DNA replication takes place in the cell centre we find that initiation of DNA replication takes place in the cell centre, such that upon initiation of DNA replication

$$d(l_i^*) = 0$$

Consequently, the y-intercept of equation (2)

$$a = -\frac{1}{2}l_i^*$$

Substituting b and a in equation (2) gives

$$d = \frac{1}{2}l - \frac{1}{2}l_i^*$$

For each copy of *oriC* we find

If $l_0 \leq l - l_i$ then $d_1^2 = -d$, and $d_2^2 = d$

If $l_i < l - l_i$ then $d_1^4 = -d$, $d_2^4 = -d(l_i) + \frac{1}{2}(l - l_i)$,
 $d_3^4 = d(l_i) - \frac{1}{2}(l - l_i)$, and $d_4^4 = +d$

4.3.2.2 Model II (fig. 4.4B)

For model II we assumed that sister copies of *oriC* move away from the cell centre together until reaching a cell length halfway between initiation and termination of DNA replication:

$$l_s = l_i^* + \frac{1}{2}(l_e - l_i^*)$$

After reaching l_s one of the copies jumps to the other side of the cell, where it continues to move away from the cell centre. Thus for each copy of *oriC*:

If $l_0 \leq l - l_s$ then $d_1^2 = d_2^2 = -d$

If $l_s < l - l$ then $d_1^2(l) = -d$, and $d_2^2 = +d$

If $l_i < l - l_s$ then $d_1^4(l) = d_2^4 = -d$, and $d_3^4 = d_4^4 = d$

4.3.2.3 Model III (fig. 4.4C)

In this model each copy of *oriC* is somewhere within the boundaries of the nucleoid(s), hence their theoretical position depends on the length of

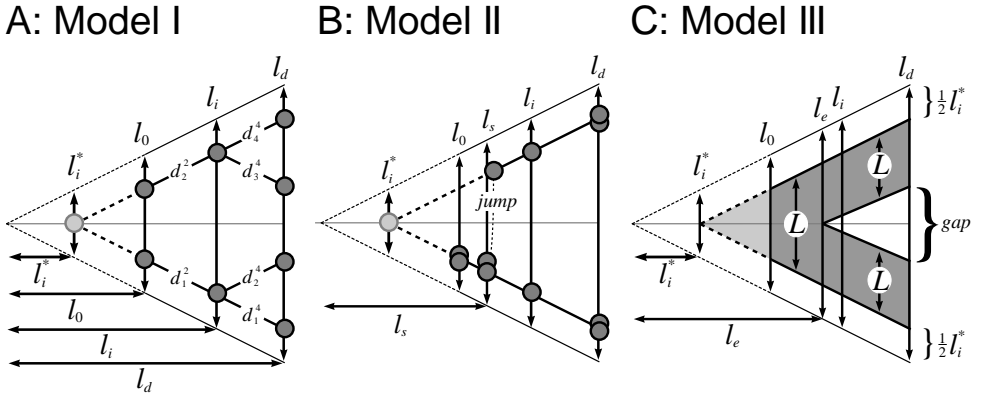


Figure 4.4 - Schematic representation of the parameters used to derive model I (A), model II (B), and model III (C)

See the appendix for descriptions of these parameters. Dotted lines represent the length of and distances in the virtual cell before cell birth.

the nucleoid(s). In the virtual cell at initiation of DNA replication, the length L of the nucleoid

$$L(l_i^*) = 0$$

which means that l_i^* represents the total length of the DNA free zones at the poles of the cell. Assuming that the length of these zones remains constant during the cell cycle we find that

$$\text{If } l_0 = l - l_e \text{ then } L = l - l_i^*$$

We assume that after termination of DNA replication two separate nucleoids appear. The length of the 'gap' between the two nucleoids in the cell centre increases until it equals l_i^* upon cell division, thus

$$\text{gap}(l) = l_i^* \frac{l - l_e}{l_d - l_e}$$

from which we derive the length of each separate nucleoid:

$$\text{If } l_e < l - l_d \text{ then } L = \frac{1}{2} \{ (l - l_i^*) - \text{gap}(l) \}$$

For each copy of *oriC* in a cell we obtain a distance value by taking a random item L_r^n out of a uniform distribution:

$$\bullet \frac{L_r^n}{L} :$$

$$\text{If } l_e < l - l_d \text{ then } d_1^2 = L_1^2 - \frac{1}{2}L, \text{ and } d_2^2 = L_2^2 - \frac{1}{2} - \frac{1}{2}L$$

$$\text{If } l_e < l - l_d \text{ then } d_1^4 = \overline{L_1^4} - \left\{ L + \frac{1}{2} \text{gap}(l) \right\}, d_2^4 = \overline{L_2^4} - \left\{ L + \frac{1}{2} \text{gap}(l) \right\}, \\ d_3^4 = \overline{L_3^4} + \frac{1}{2} \text{gap}(l), \text{ and } d_4^4 = \overline{L_4^4} + \frac{1}{2} \text{gap}(l)$$

The second term of each d_r^n places the cell centre at position 0 (the origin), assuming that nucleoids are symmetrically positioned in the cell.

4.3.3 FISH labelling efficiency

The probability of a cell to have r out of n copies of *oriC* labelled is

$${}_r^n P = \binom{n}{r} \cdot p^r \cdot (1 - p)^{n - r} \quad (3)$$

in which p is the probability of a single DNA region to be labelled. The probability to have at least one copy of *oriC* labelled in a cell is defined by

$$r > 0, {}^n P = {}^2 P \cdot ({}^1 P + {}^2 P) + {}^4 P \cdot ({}^1 P + {}^2 P + {}^3 P + {}^4 P) \quad (4)$$

which can be estimated by scoring of the fraction of cells with at least one focus out of 100 randomly selected cells. ${}^2 P$, and ${}^4 P$ are the probabilities of a cell to contain two or four copies of *oriC* respectively. We assume that the number of cells in a steady-state population is described by

$$N(l) = N_0 2^{-\frac{l - l_0}{l_1}} \quad (5)$$

from which we derive ${}^2 P$ by integrating (5) between l_0 and l_1 .

Consequently ${}^4 P = 1 - {}^2 P$. By substituting in equation (4) each ${}^n P$ by

equation (3), and the estimates of ${}^n_{r>}P$, 2P , and 4P we can derive p . The experimental values of the model parameters for *oriC* were ${}^n_{r>}P = 0.5$ (Roos *et al.*, 2001), ${}^2P = 0.8$, and ${}^4P = 0.2$; consequently $p = 0.3$.