Modeling Control of DNA-Replication in Bacterial Cells

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Summary

We have developed a dynamic model of DNA replication control in bacterial cells and of the subsequent cell division. The main hypothesis is that a certain protein, which has a negative feedback regulation on its own production, is an essential factor in initiation of the replication. The model is stochastic in the sense that the kinetic association and dissociation processes are assumed to take place in accordance with a Poisson probability distribution with mean values that match experimentally determined constants. An important feature of our model is that it shows correlation between the magnitude of the kinetic rate constants and the size, stability and the dynamics of the cell. The model thus allows us to analyze the distribution of cell volumes at the time of initiation for different growth rates, different association and dissociation constants, and different promoter strengths.

Introduction

Extensive studies have been performed to reveal the dynamics of bacterial cell division (Ingraham *et al.*, 1983). Particular interest has been addressed to *E. coli*, a bacteria which has been studied in many different connections and for which more than 1000 genes are well characterized (Bachmann 1983). For several of these genes, information is available about nucleotide sequence and protein function. For many of the central genes involved in the growth control of *E. coli*, very detailed experiments concerning this regulation have been performed. These include studies of (i) the replication of DNA which ensures heredity and is tightly coupled to the control of cell growth and cell division (Meyenburg *et al.* 1987), and of (ii) the system for gene expression including ribosomes and RNA polymerase (Ingraham *et al.* 1983). Typically, these phenomena have been examined on the single gene level, although it is clear that a very high degree of interaction is necessary to make the cell work. In order to understand such a system, models involving several interacting control

loops would be of great help.

In our work we have focused on the initiation of replication of the DNA molecule inside the bacterial cell. We have chosen to consider only a few components that are believed to be essential for this process, rather than including many different substances in an attempt to create a detailed model of a bacterial cell as a whole.

Early physiological measurements implicated a model proposed by Donachie (1968) which essentially states that replication is initiated when a certain ratio between replication origins and cell mass is reached. This ratio would be constant for wide range of growth conditions. However, at that time Donachie was not able to explain how this control took place. In the following years several different control mechanisms were proposed (reviewed by Meyenburg *et al.* 1987), although none of them seemed to be satisfying. Many experiments pointed to the dnaA protein as being the key mediator in the initiation of DNA replication. Inspired by a model suggested by F. G. Hansen (personal communication), we here formulate a detailed, dynamical and stochastic model which can explain many of the published experiments concerning initiation control of DNA replication.

In the following we shall first give a an overview of the model followed by a presentation of some simulation results.

The model

The molecular components of the model are:

1) dnaA protein

2) dnaA boxes

3) dnaA promoters

4) dnaA-mRNA

The number of molecules of each of these components is represented by a state variable. In addition, the cell volume is a state variable of our model.

1) The amount of free dnaA protein is assumed to determine when initiation of replication of the DNA-molecule takes place. In the model, this occurs if a certain number of dnaA proteins are present in a free (unbound) state inside the cell. The amount of free dnaA proteins varies, partly because a dnaA protein can bind to the dnaA-boxes (Fuller *et al.*, 1984).

2) The dnaA-boxes are sites (sequences) on the DNA molecule with the consensus sequence $TTAT^{C}_{A}CA^{C}_{A}A$ (Fuller *et al.* 1984). Each site allows one dnaA protein to bind. The binding of dnaA protein to the boxes is determined by the rates of association and dissociation, the former being dependent on volume. Some of these boxes are close to *oriC*, the location on the DNA molecule where the replication starts. An additional number of boxes are distributed along the DNA molecule. The reaction is:

$$\mathrm{dnaA_{free}} + \mathrm{box_{free}} \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} \mathrm{dnaA_{bound}} \qquad (1)$$

Here $dnaA_{free}$ represents the free dnaA proteins, and $dnaA_{bound}$ molecules bound to dnaA boxes. K_1 (liter/mole/sec) and K_{-1} (sec^{-1}) are the corresponding association and dissociation constants. The rates of association and dissociation are given by:

$$R_{ass} = K_1 \cdot [dnaA_{free}] \cdot [box_{free}] \quad (2)$$

$$R_{diss} = K_{-1} \cdot [dnaA_{bound}] \quad (3)$$

where R_{ass} and R_{diss} are given in mole/liters sec^{-1}. Square brackets represent concentration. The equilibrium constant is given by:

$$K_m = K_{-1}/K_1 = dnaA_{free} \cdot box_{free} / dnaA_{bound} \cdot vol \cdot N_A \quad (4)$$

N_A is Avogadros number: $6.023 \cdot 10^{23}$ (mol^{-1}), and vol is the cell volume.

3) The production of dnaA protein is regulated by a negative feedback mechanism. The rate of transcription is determined by the strengths of the two dnaA promoters, P_1 and P_2. It has been shown that the transcription of dnaA is regulated by the dnaA protein, and experimental evidence suggests that only P_2 is regulated in such a way that when dnaA protein is bound to the dnaA box between P_1 and P_2, then P_2 is closed (Atlung *et al.* 1985, Braun *et al.* 1985). The rate of transcription is given by:

$$R_{trans} = R_1 + R_2 \cdot H \quad (5)$$

where R_1 and R_2 are the transcription rates for the two promoters, and H is either 0 or 1. We assume that $R_1 = 0.15 \cdot R_2$ (Atlung *et al.* 1985).

4) From the two promoters mRNA coding for dnaA-protein is produced and subsequently translated into dnaA protein. mRNA molecules are degraded down with a half-life of approximately 1 min.

In the model, the cell volume grows exponentially with time, independent of all other processes except division of the cell which causes the volume to be halved.

The model is **stochastic** in the sense that all transformations are assumed to take place in accordance with Poisson probability distributions. Thus, the binding of a dnaA protein to a dnaA box, for instance, happens with a certain probability. Since the amount of each substance often is very small, this stochastic approach is preferable to a continuous description. Small fluctuations in number can have a very dramatic effect.

With the initiation of DNA replication, a series of events is commenced:

At the same time that a box is replicated, bound dnaA protein is released. After replication of a

sequence on the DNA, a certain period of time passes before this sequence becomes active again. During this period of time (5 min), the processes of methylation, supercoiling and folding take place (Ogden *et al.* 1988). With respect to the model, this means that no dnaA protein can bind to a box before it has gone through these processes, and that no initiations of replication can take place. However, after the sequences that make up *oriC* have been methylated etc., the next initiation may occur before the former one is terminated. This makes it possible to obtain doubling times shorter than the time it takes to copy the chromosome: several replications will take place simultaneously. The time it takes to replicate a DNA molecule (C-time) and the following time until the cell division (D-time) is here set to a constant of 60 minutes (Helmstetter 1987). At the cell division, the volume is split equally between the two daughter cells, and, of course, each gets its own DNA molecule. The dnaA protein and mRNA molecules are divided stochastically between the two according to a binomial distribution. Only one daughter cell is followed in it's further development.

Results of simulations.

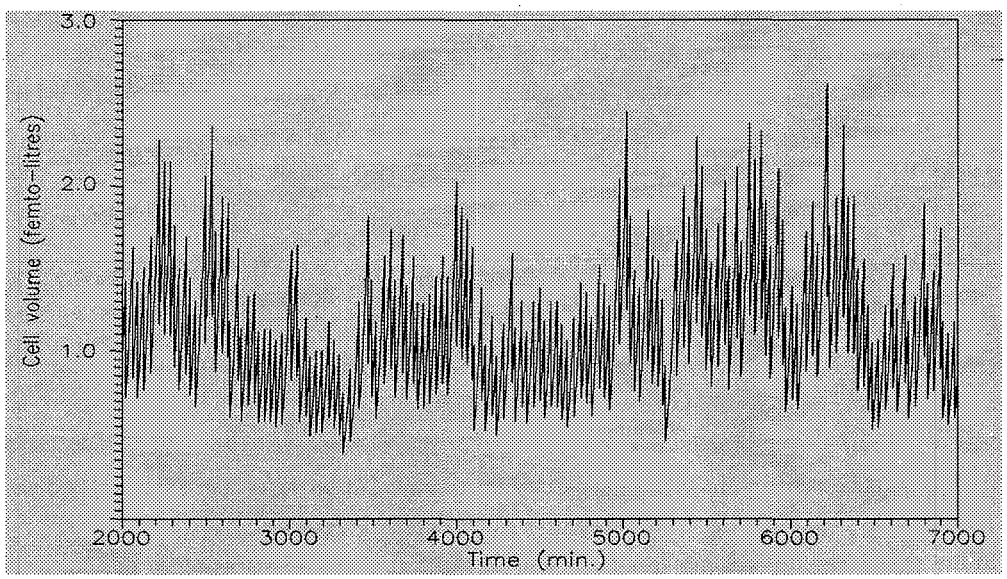

Fig. 1 The cell volume as a function of time

The simulation showed in fig. 1 follows the volume of a single cell through many cell divisions. The volume of the cell is well controlled: large cells give rise to smaller ones and the smaller cells approach larger ones. The average volumes at cell division and at initiation of DNA replication are about 1.5 10^{-15} liters and 1.0 10^{-15} liters, respectively and both have a standard deviation of 14 - 18 %. For the cell-age the standard deviation is about 25 %. This all agrees with earlier experimental results, where the volume at division was found to vary 9 - 15 % and the life-length varies 22 - 27 % (Koch 1977).

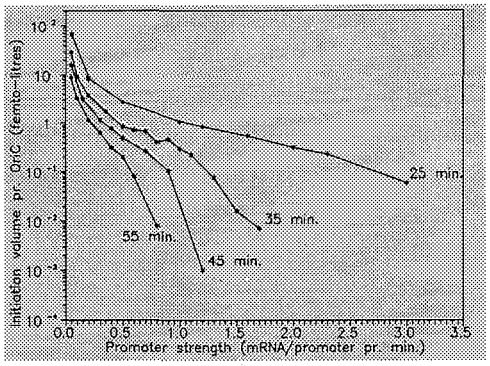

Fig. 2 The initiation volume as a function of the promoter strength for four different doubling times

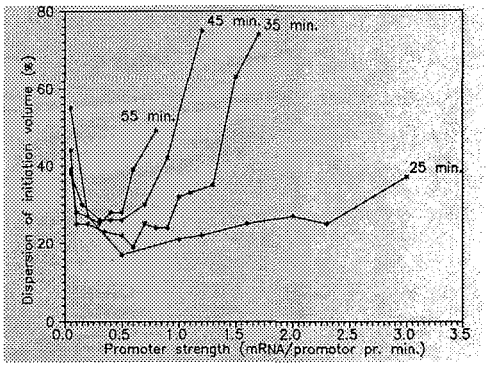

Fig. 3 Dispersion of the initiation volume in per cent of the average initiation volume of the individual cell as a function of the association constant.

If the simulations are performed with different values for the production rate of dnaA protein (promoter strength), it turns out that the model only gives stable results within a limited range of promoter strengths. When the promoter strength falls outside of this range, the cell is unable to regulate its volume satisfactory: either very small or very large cells appear during the simulations. In order to quantify this effect, we have plotted the average cell volume at initiation and the standard deviation of this volume as functions of promoter strength (fig. 2 and 3). It is clearly seen that as the promoter strength approaches zero or becomes greater than 0.5 - 1.0, the volume control is lost. This is qualitatively explained by the fact that increasing promoter strength means that a dnaA promoter should be less open to produce the same amount of dnaA protein, hence R_{ass} on the average has to be higher. This is obtained by decreasing the volume.

We have examined why there is an increase in dispersion when the promotor strength becomes too high (>1 mRNA/promoter/min) or too low (<0.1 mRNA/promoter/min) (fig. 3). We found that a regression line of the initiation volumes had a slope that differed from zero. Such that the initiation volume increased (promoter strength<0.1 mRNA/promoter/min) or decreased (promoter strength>1 mRNA/promoter/min) through the entire simulation causing the cell volume to be destabilized.

The cell is destabilized when the promoter strength is lower than 0.1 mRNA/promoter/min, because neither the regulated nor the unregulated promoter can produce enough dnaA protein in a cell cycle. When the promoter strength is too high (>1.0 mRNA/promoter/min) the unregulated promoter alone produces more dnaA than required; the regulated promoter is never open, and the cell divides before it is sufficiently large.

Similarly it turns out that a higher association constant for the dnaA protein binding to dnaA boxes has a marked effect on the stability of the volume control (fig. 4 and 5).

When the volume is low, the rate of association will be relatively high and this results in a decrease in the production of dnaA protein. There is always a certain level of dnaA that have to be produced before initiation and the time between initiations will therefore increase. This leads to an increase in the volume.

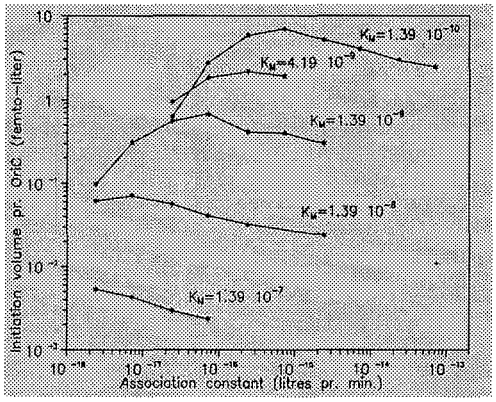

Fig. 4 Initiation volume as a function of the association constant. K_m is the equilibrium constant.

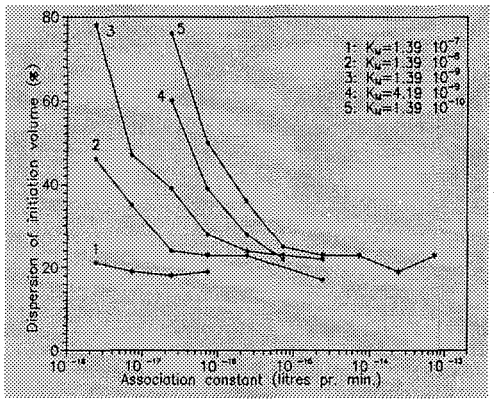

Fig. 5 The dispersion of the initiation volume in per cent of the average initiation volume of the individual cell as a function of the association constant.

In fig. 4 we see that each curve has a maximum. When the association constant is lower than this value, the dispersion of initiation volume slowly increases. This increase may be explained by the fact that the dissociation constant from boxes is very low, the lifetime of the complex is longer than the cell cycle. This makes the "reaction time" of the control mechanism too slow, it cannot respond to changes or fluctuations within a cell cycle.

Changing the equilibrium constant K_m leads to a reciprocal change in initiationn volume. From equation (2) it is seen that when K_m is changed (K_{-1} is kept constant) the volume has to follow if the other values are unchanged. It is observed that the reaction is not unambiguously decided by the K_m value, but is dependent on association and dissociation rates as well. This example shows that equilibrium kinetics is not sufficient to model the processes involved in the initiation control.

In fig. 6. the amount of free dnaA protein molecules are shown along with the total amount of dnaA protein and cell volume as a function of time. Most of the time there is about 5 molecules of dnaA protein in a cell although this number does fluctuate a lot, between 0 and 10, this was also found by Mahaffy and Zyskind (Mahaffy *et al.* 1989). At irregular intervals peeks of free dnaA molecules are seen this corresponds to the initiation of DNA replication. The amount of total dnaA protein

seems to be approximately proportional with cell volume. It is important to realize that fluctuations in the number of free dnaA molecules easily can initiate a DNA replication, the stochastic aspect here is very important, however if the initiation is early in one cell cycle there will be a probability of it being correspondingly late in the next cell cycle.

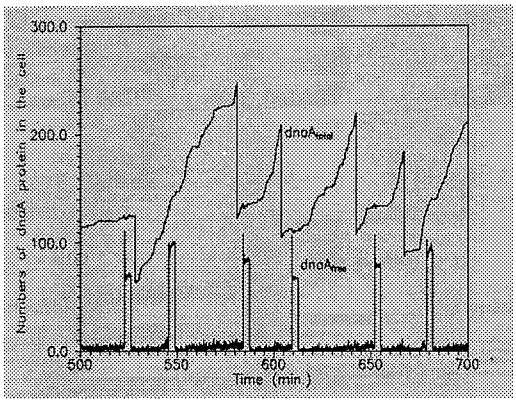

Fig. 6 The amount of dnaA protein as a function of time.

Conclusion

The model presented here differs in two important aspects from previously publiched models (Margalit *et al.* 1984, F. G. Hansen personal communication, Mahaffy *et al.* 1989). In the first place, the model is dynamic, and we have shown that the association rate for the binding between dnaA protein and dnaA boxes is important. Secondly is that our model is stochastic in all the variables where small numbers are likely to occur. It is possible to test our prediction of the stability of cell volume regulation as a function of binding kinetics and promoter strength. Especially one could imagine that *dnaA* mutants exist which have different binding kinetics.

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