Simulation of the Prokaryotic Cell Cycle at http://simon.bio.uva.nl/cellcycle/

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ABSTRACT

The bacterial Cell Cycle is presented using the Simulation program CCSim, which employs four parameters related to time (inter-division τ, replication C, division D) and size (mass at replication initiation Mi), sufficient to describe and compare bacterial cells under various conditions. The parameter values can be altered and the effects of the alterations can be seen. CCSim is easy to use and presents the kinetics of cell growth in a digestible format. Replicating chromosomes and growing bacillary cells are coupled to parameters that affect the cell cycle and animated. It serves as an educational tool to teach bacteriology and to compare experimental observations with the model best describing cell growth thus improve our understanding of regulatory mechanisms. Examples are displayed of transitions between known physiological states that are consistent with experimental results, including one that explains strange observations. The program predicts enhanced division frequencies after a period of slow replication under thymine limitation if a minimal distance (Eclipse) exists between successive replisomes, as observed. Missing features and ways to improve, extend and refine CCSim are proposed, for both single cells and random populations under steady-states of exponential growth and during well-defined transitions. Future improvements and extensions are proposed for single cells and populations.

Keywords: The Bacterial Cell Cycle, Coupling between Chromosome Replication and Cell Division, Temporal and Spatial Regulation, Duplication of the Nucleoid and the Sacculus, *in silico* Simulation, Testing Predictions, Initiation Eclipse, Thymine Limitation.

INTRODUCTION

The coupling between chromosome replication and cell division includes *temporal* and *spatial* elements. In bacteria, these have globally been resolved, but the details and action mechanisms are still obscure. Crucial links are thus sought between duplications of the nucleoid (DNA) and the sacculus (murein), the only unique macro-molecular structures.

Many bacterial species multiply in aqueous salts solution at rates μ that depend on nutritional conditions [11, 15]. The shortest doubling time τ for the model species *Escherichia coli* at 37ºC in rich media is about 20 min (equivalent to $\mu = 3$ hr^{-1}). Irrespectively, the time C taken to replicate its 4.6 Mega-base pairs chromosome is usually about 40 min at values of τ up to about 70 min (μ down to 0.9 h^{-1}) [7]. The cell divides to two practically identical daughters [18] a time $D \approx 20$ min after replication-termination. Taken together with other basic data [9, 11, 15] resulted in the conclusion that cell mass at the time of replication-initiation, Mi is roughly constant per *oriC* [1, 14]. Growing at an exponential rate, the cell thus divides ($C+D$) min after initiation with a mass of $Mi \times 2^{(C+D)/\tau}$. The changing exponential rate of cell growth at varying media is not matched by changes in the linear replication rate; they are however coupled by frequencies: the frequency of initiations adjusts to that of mass doublings. The values of C, D and Mi change only slightly with τ [5, 12, 19, 23], and can be manipulated by genetic or environmental means [10, 16]. Many of these conclusions were based on experimental findings with a method for cell cycle analyses of minimally-disturbed, exponentially-growing *E. coli* cultures [6], and have been confirmed by numerous observations in other eubacteria [7].

Multi-forked replication, for example, was exhibited in fast growing cells, consistent with results obtained several years earlier by genetic transformation in the Gram-positive model species *Bacillus subtilis* [17].

Upon transfer of an exponentially growing culture to a richer medium (so-called nutritional shift-up), the ordered series of changes in the rates of macromolecular syntheses culminates in increased cell division rate after (*C*+*D*) min in the so-called "rate maintenance" phenomenon [9, 11]. (And see details below.)

CELL CYCLE SIMULATION

The user-friendly program CCSim that is posted at http://simon.bio.uva.nl/cellcycle/ pictures semi-realistically and schematically the relationships between replication of the chromosome, cell mass growth and division within the framework of the Cooper-Helmstetter model [7]. It is useful for students at the under-graduate and graduate levels as well as to scientists who investigate various aspects of the bacterial cell cycle [7a, 10].

CCSim exploits the four parameters (τ, *C*, *D* and *Mi*) to follow single cells during exponential growth and transitions such as nutritional shifts [9, 11] and other changes in parameters as may be expressed in mutants or under experimental conditions that modify *C* [21], *D* [12] or *Mi* [5, 19, 23]. Its default values are (60, 40, 20, 1) respectively (Fig. 1), but they can easily be modified at will in steps of 1 min (and 0.01 for the latter).

The main window displays changes with time in cell mass (black line, *M*, in *Mi* units [5]) rising exponentially, in DNA contents (magenta, *G*, in genome equivalent units [7]) increasing linearly, and the resultant, fluctuating DNA concentration (green, *G/M* [13, 21]) at a resolution of 1 min. The first two parameters change abruptly at cell division, the rates of the last two (rise or drop, respectively) change at the times of initiation and termination [7], and each can be removed by clicking off in the appropriate box below. The plot can be run manually (by moving the bar below) or automatically (by pressing the Run button once, or twice to stop), animated and reset at will. The red and blue horizontal bars represent *C* and *D* time intervals respectively, with a split in the blue (during the *D*) when the two daughter nucleoids segregate *S* min after termination of replication [8].

Fig. 1. The default picture of CCSim as seen upon entry to http://simon.bio.uva.nl/cellcycle/ (freely available).

In parallel windows of the screen, the following additional pieces of information are displayed:

- Values of all parameters and measures mentioned above (and more) - on the top left hand side as they change at 1 min intervals (0.5 min during the automatic run).

- The state of the circular chromosome (or optional, linear half-chromosome) with *oriC*'s (red dots), replisomes (black) and *terC*'s (blue) at a 70º angle. (An option exists to change the observation angle.)

- Links to a brief Explanation of the bacterial cell cycle, an extensive Glossary and Printing options are displayed above.

- Two series of 5 bars, left set for pre-change parameters and right for post-change, which can easily be manipulated within a wide range of values (reflecting experimental conditions).

- A symbolic cell with its nucleoid as they grow and constrict to two. The scheme lacks several features, to be improved in later versions of the program: it does not indicate the realistic change in cell width with growth rate, it does not follow exact exponential elongation with time, and it is concealed during transitions, while exponential growth has not been reached; the missing items are still to be well defined.

- Four optional "cases" are given automatically, the 4th includes an Eclipse—a minimal possible distance between two successive replisomes [20, 26], where *E* limits the number of replisones to a maximum as described below. The command "Swap Parameters" mirrors the values of each of the 5 scrollbar pairs, interchanging the situation three complete cycles before the transition and long afterwards.

It is useful to study this setup by steps, from simpler conditions to increasingly complicated ones, as follows [7]:

(a) $\tau > C+D$ Cell cycles do not overlap (i.e., *B* [$\equiv \tau - (C+D)$] is analogous to the eukaryotic G1 (green bar in the simulation), *C* to S, and *D* to G2);

(b) $C+D > \tau > C$ *B* disappears, and *Mi* is reached at the latter part of mother's cell cycle, during the *D* period;

(c) $C > \tau > C/2$ Initiation of replication occurs before the previous cycle has terminated, i.e., replication cycles overlap;

(d) $C/2 > \tau > C/3, C/3 > \tau > C/4$, etc The degree of overlap is increasingly complicated and the number of replisomes rises exponentially with $n = C/\tau$;

(e) Same as (c) or (d) but with an *E* that varies at will, as explained below.

Several remarks are noteworthy and instructive:

- In exponentially growing cultures [3] both cell mass, $M = \ln2 \times Mi \times 2^{(C+D)/\tau}$ and DNA content, $G = \tau \times [2^{(C+D)/\tau} - 2^{D/\tau}]/C \times \ln2$ rise with growth rate (shortened doubling time τ). Concentration of DNA, $G/M = \tau \times (1 - 2^{-C/\tau})/Mi \times C \times \ln2$, decreases because the former rises faster. The same happens at slower replication rates (extended *C* values). The lowest limit of *G/M*

required for a cell to normally survive and multiply is yet to be determined experimentally.

- M rises and drops with analogous changes in Mi, but *G* remains the same due to the linear nature of chromosome replication and the constant time from initiation to division (*C+D*).

- The meaning of *D* has not yet been explained in molecular terms; it was empirically defined as the difference between (*C+D*) and *C*, and the existing, feasible explanation [25] to its constant value must still be substantiated.)

- The well-defined perturbation that has extensively been studied is the nutritional shift-up, at which an exponentially growing culture [3] is transferred into a richer medium that supports faster growth rate. An ordered series of changes in rates of macromolecular syntheses upon such a change culminates in change of cell division rate after (*C+D*) min in the so-called "rate maintenance" phenomenon that puzzled the community for a decade since its discovery [9, 11]. This is simulated by setting $\tau_2 < \tau_1$, as exemplified in Fig. 2:
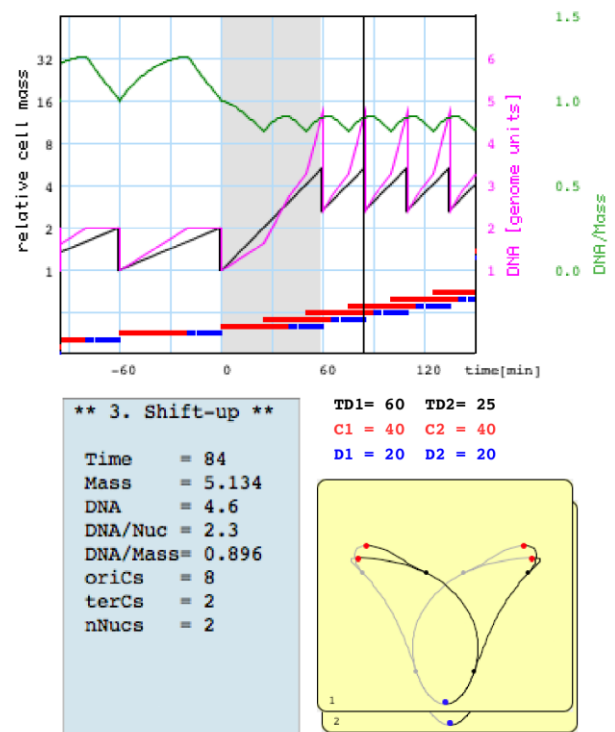

Fig. 2. An example of nutritional shift-up, generated using CCSim. A newborn cell in an exponentially growing culture under steady-state conditions [3] is followed before and after a shift-up from $\tau_1 = 60$ min to $\tau_2 = 25$ min.

THYMINE LIMITATION AND ECLIPSE

The changes after nutritional shift-up leading to the new steady-state do not occur abruptly, and hence the real situation is slightly more complicated than described above. The change in growth rate is relatively slow because the profile of gene expression changes according to the nature of the nutritional shift [9, 11], sometimes even dramatically. This is why the other perturbation, changing the replication time *C* by

manipulating the concentration of the specific DNA precursor, thymine supplied to *thyA* mutants [13, 21, 25], resolves this difficulty, resulting in an abrupt change: no such qualitative change is anticipated or observed. However, the predicted behavior (Fig. 3A), reaching a steady-state after $(C+D+\tau)$ min, is only found at relatively slow growth rates (e.g., in glycerol, with $\tau = 60$ min) [12, 21, 25].

At faster growth rates (e.g., $\tau = 40$ min), the divisions of such thymine limited cells are delayed without affecting the mass growth rate [13, 21, 22, 24-27]. Consequently, the cells continuously enlarge, and their mass increase is accommodated by widening, then elongating and later reaching monstrous shapes and dimensions. Under such conditions, the culture does not reach a steady state but rather grows in a mode defined as 'normal' [3].
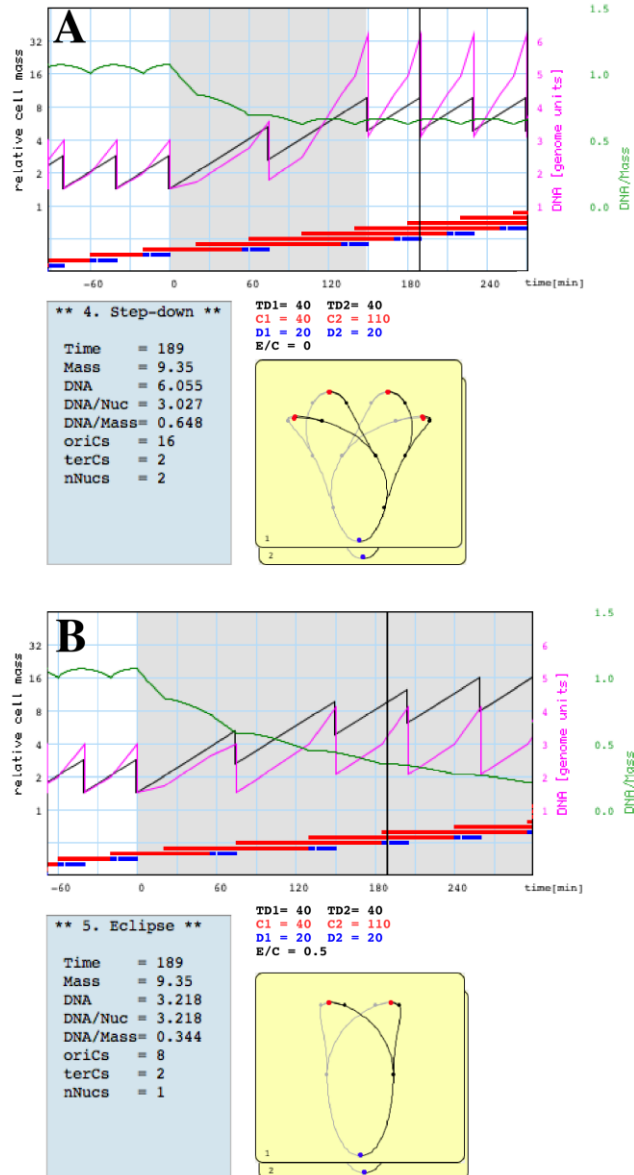
```
** 4. Step-down **        TD1= 40   TD2= 40
                          C1 = 40   C2 = 110
   Time    = 189          D1 = 20   D2 = 20
   Mass    = 9.35         E/C = 0
   DNA     = 6.055
   DNA/Nuc = 3.027
   DNA/Mass= 0.648
   oriCs   = 16
   terCs   = 2
   nNucs   = 2
```

```
** 5. Eclipse **          TD1= 40   TD2= 40
                          C1 = 40   C2 = 110
   Time    = 189          D1 = 20   D2 = 20
   Mass    = 9.35         E/C = 0.5
   DNA     = 3.218
   DNA/Nuc = 3.218
   DNA/Mass= 0.344
   oriCs   = 8
   terCs   = 2
   nNucs   = 1
```

Fig. 3. An example of a thymine step-down, generated using CCSim. A newborn *thyA* cell in a culture growing exponentially under steady-state with $\tau = 40$ min before and after a step-down from conditions supporting $C_1 = 40$ min to $C_2 = 110$ min, without an eclipse ($E = 0$) (**A**) and with $E = 0.5$ (**B**).

This behavior, depicted in Fig. 3B, has been explained by existence of an Eclipse—a minimal possible distance l_{min} between two successive replication forks (replisomes [17]), which limits the number of replisomes to a maximum that depends on that presumed distance relative to the total half-chromosome length $L_{0.5}$, i.e., $l_{min}/L_{0.5}$. If this limit is smaller than 0.5 (quarter L) [26], it is never reached under normal conditions because the minimal doubling time achievable at 37°C $\tau_{min} = 20$ min (and $C = 40$ min), hence the maximum replisomes number is 3 (two so-called 'positions' n [$= C/\tau$], calculated by 2^n-1) [17].

Under these circumstances (fast growing cells with slow replicating chromosomes, where the minimum distance between successive replicating positions is breached), G remains constant but G/M drops with time at a rate that depends upon the difference ($C/\tau - L_{0.5}/l_{min}$) - the number of positions required to maintain a steady state growth (C/τ) minus the maximum number of positions possible ($L_{0.5}/l_{min}$) [26].

Case (e) can thus explain the formation of such monstrous cells (Fig. 4) during a long evolution [21, 22, 24-27]. This explanation must obviously still be further substantiated.

Fig. 4. Examples (in various scales) of bizarre-shaped, thymine-limited cells. Left: branched, adapted from [22]; middle: DAPI-stained, unpublished; right: with polar constriction (yellow arrow), from [27].

RESTORING THE ECLIPSE DEFICIENCY

The explanation (Fig. 3B) given here to the apparently bizarre observations ([21], Fig. 4) was recently tested experimentally; enhanced frequencies of synchronous cell divisions that followed an interval of reduced rate of DNA replication [27] is consistent with the notion of eclipse [2, 4, 20, 26] and serves as an example of the usefulness of CCSim. The 'deficit' in cell divisions due to progressive, delayed initiation events (Fig. 3B) is predicted to be restored as soon as the rate of replication returns to normal by stepping up the concentration of thymine in the growth medium ([13, 21], Fig. 5). Moreover, running CCSim with the appropriate parameters predicts a transition period of at least 5 divisions at a frequency (every 20 min; Fig. 5) that is about twice that of mass doublings ($\tau = 40$ min), a prediction that was experimentally fulfilled (Fig. 6). The five almost simultaneous divisions observed in the batch culture can currently not be predicted by CCSim until it is extended to populations, as proposed in [27].
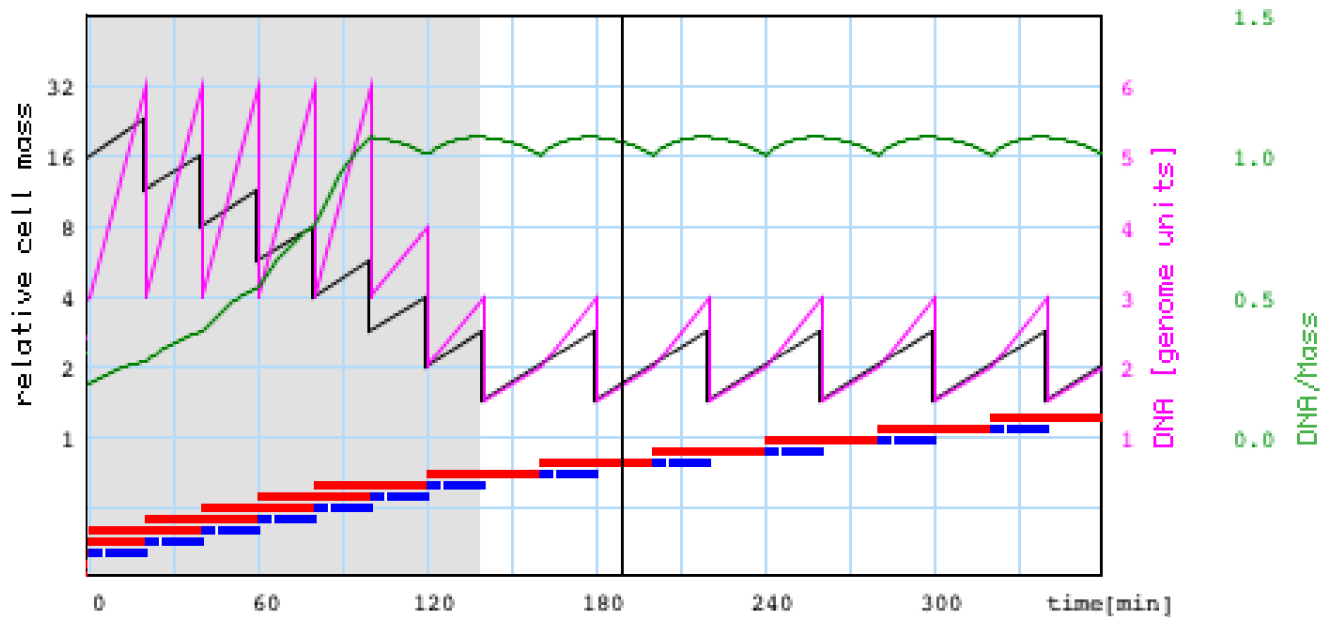
Fig. 5. A glucose-grown ($\tau = 40$ min) cell, stepped-up after 7 hrs growth in conditions of $C = 100$ min to normal $C = 40$ min, with an eclipse E of 0.5. Adapted from Fig. 4B at [27].
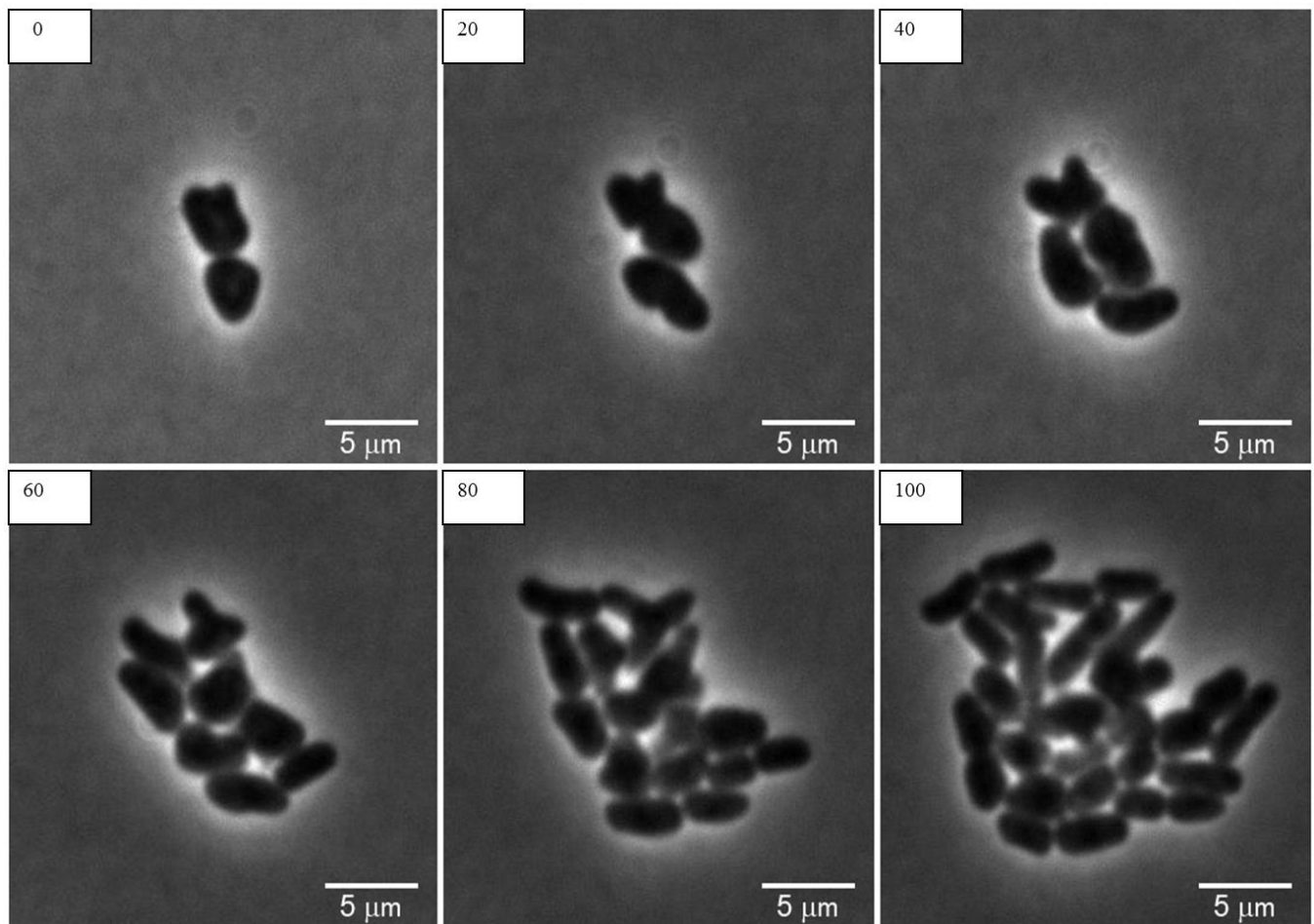

Fig. 6. Selected pictures (minutes, at top left corners of panels) from a time-lapse film. Adapted from [27].

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FUTURE EXTENSIONS OF CCSim

Similar tests with mutants such as *dnaAts* may help to decipher the mechanism that regulates the complex pathway leading to initiate a round of chromosome replication (e.g., [7a, 10]).

The CCSim program lacks several features, to be advanced in forthcoming years, as follows: (a) The schematic, symbolic cell is not drawn in proportion to size or dimensions under different growth conditions, nor at an exponential rate of elongation, and is concealed during transitions; (b) Behavior of cell populations in batch must take into account the degrees of variation among the individual cells in doubling time, size and division symmetry; (c) The empirical period *D* remains enigmatic and a molecular explanation must still be elaborated; (d) Species with linear or multiple chromosomes and different physiology such as *Bacillus*, *Mycobacterium*, *Caulobacter* and *Vibrio*, will need elaborations by additional parameters in specific sections.

The general idea is for CCSim to provide a new means by which the bacterial cell biology can be better understood using a powerful though friendly tool that allows rigorous testing of innovative hypotheses with specific predictions.

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