Review

Instructive simulation of the bacterial cell division cycle

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The coupling between chromosome replication and cell division includes temporal and spatial elements. In bacteria, these have globally been resolved during the last 40 years, but their full details and action mechanisms are still under intensive study. The physiology of growth and the cell cycle are reviewed in the light of an established dogma that has formed a framework for development of new ideas, as exemplified here, using the Cell Cycle Simulation (CCSim) program. CCSim, described here in detail for the first time, employs four parameters related to time (replication, division and inter-division) and size (cell mass at replication initiation) that together are sufficient to describe bacterial cells under various conditions and states, which can be manipulated environmentally and genetically. Testing the predictions of CCSim by analysis of time-lapse micrographs of *Escherichia coli* during designed manipulations of the rate of DNA replication identified aspects of both coupling elements. Enhanced frequencies of cell division were observed following an interval of reduced DNA replication rate, consistent with the prediction of a minimum possible distance between successive replisomes (an eclipse). As a corollary, the notion that cell poles are not always inert was confirmed by observed placement of division planes at perpendicular planes in monstrous and cuboidal cells containing multiple, segregating nucleoids.

Growth, chromosome replication and cell division – coupling and dissociation

A wild-type prototrophic bacterium such as the Gram-negative species *Escherichia coli* can synthesize all of the component macromolecules that are necessary for duplication from aqueous salts solution. In such media, the multiplication rate depends on the carbon source, the most efficient of which is glucose, supporting a doubling time τ of about 40 min at 37 °C. Slower rates are obtained with poorer carbon sources, and addition of organic building blocks results in faster rates; the maximum achievable is about 3 h^{-1} (i.e. $\tau_{min} \approx 20$ min) (Maaløe & Kjeldgaard, 1966; Schaechter *et al.*, 1958). Irrespective of the actual growth rate, a cell divides into two morphologically identical daughters (Trueba & Woldringh, 1980) about 20 min (designated D) after its chromosome has terminated replication; and since the time C taken for a replisome to complete duplication of the entire genome (of some 4.6 Mb) is also constant, approximately 40 min,

cell division follows initiation of replication by about 1 h. This idealistic picture, the so-called Cooper–Helmstetter model (Helmstetter *et al.*, 1968), was visualized in *E. coli* by the then novel method (the 'baby-machine') of achieving minimally disturbed synchronous cells (Helmstetter & Cummings, 1964). The model was confirmed for cells growing with doubling times τ ranging from 20 to 70 min (growth rates, μ, of 3 to 0.9 h^{-1} respectively), and multi-forked replication was demonstrated in fast-growing cells, consistent with results obtained several years earlier by genetic transformation in the Gram-positive model species *Bacillus subtili*s (Sueoka & Yoshikawa, 1965). The model survived over 40 years with minor modifications of parameter values (e.g. Bipatnath *et al.*, 1998; Michelsen *et al.*, 2003) because it took into consideration all that was known when published in 1968 (Helmstetter *et al.*, 1968). Many of its conclusions have since been confirmed in other eubacteria (Helmstetter, 1996), albeit with mechanistic variations (e.g. Toro & Shapiro, 2010) and hence it can be termed as 'The Central Dogma of the Bacterial Cell Division Cycle' (BCD). The BCD dogma relates to the concept that the linear replication of the genome, which initiates every doubling in cell mass, must terminate some time before the cell divides in two – otherwise species

A supplementary video is available with the online version of this paper.

survival cannot be ensured. The values of the parameters involved are immaterial for this concept to hold; they differ between species and can be varied by environment, mutations and drugs. Similarly, the specific mechanism by which the cell regulates these processes and the necessary coupling between them is irrelevant here.

This sequence of events and processes described by the model (Helmstetter et al., 1968) is subject to changes in a variety of mutants and by drugs that affect the multitude of reactions involved (Slater & Schaechter, 1974). It explains the change of cell size and macromolecular composition at different growth rates and predicts most observations under steady-state growth perturbations such as nutritional shifts (Maaløe & Kjeldgaard, 1966; Kjeldgaard et al., 1958) provided that the initiation event occurs, to a first approximation, every doubling in cell mass. Integrating the results for constants C and D (Helmstetter et al., 1968) with the way cell mass changes with growth rate, described a decade earlier (Schaechter et al., 1958), yielded the elegant outcome that cell mass at initiation of chromosome replication Mi is roughly constant per oriC (Donachie, 1968; Pritchard et al., 1969). The complex mechanism regulating this crucial event in the life cycle of a cell, initiation of chromosome replication, is under intensive investigation (Leonard & Grimwade, 2010), but this apparently constant ratio (between Mi and the number of replication origins oriC at initiation) is a useful parameter. The cell cycle ends $(C+D)$ minutes later, when cell mass reaches $Mi \times 2^{(C+D)/\tau}$. The changing exponential rate of cell growth in varying media is thus not matched by similar changes in the linear replication rate; they are however coupled by frequencies: the frequency of initiations follows that of mass doublings. One should keep in mind that these values and constants are not 'sacred': they do change with τ, more so at slow growth rates (Zaritsky & Zabrovitz, 1981; Wold et al., 1994), and can be manipulated by various means (e.g. Meacock & Pritchard, 1975; Wold et al., 1994). Nevertheless, the BCD dogma has been exceedingly helpful in disclosing interesting phenomena (e.g. Zaritsky et al., 2006, 2007).

The well-defined perturbation that has extensively been studied is the nutritional shift-up, in which a steady-state culture is transferred into a richer medium that supports faster growth. An ordered series of changes in rates of macromolecular syntheses upon such a change culminates in an increased cell division rate after $(C+D)$ minutes in the so-called 'rate maintenance' phenomenon that puzzled the community for a decade following its discovery (Kjeldgaard et al., 1958). It must be borne in mind though that the changes leading to the new steady-state growth are not occurring abruptly, and hence the real situation is complicated. The profile of gene expression changes according to the nature of the nutritional shift (Maaløe & Kjeldgaard, 1966), sometimes even dramatically, causing a relatively long period of adaptation before a new steady-state growth is reached.

The dissociation between the rates and modes of mass synthesis and chromosome replication, first described by modifying the former under a variety of nutritional conditions (Helmstetter et al., 1968), was later demonstrated by manipulating the latter using different concentrations of the specific precursor thymine in the medium of thymine-requiring strains (Pritchard & Zaritsky, 1970). This method is more amenable to accurate analysis because the change is imposed immediately upon stepping the thymine concentration up or down, without affecting the multitude of metabolic pathways and interactions between them prevailing in the cell as do nutritional shifts (Maaløe & Kjeldgaard, 1966). Various studies (reviewed by Zaritsky et al., 2006) confirm this prediction. To the best of current knowledge, thymine is incorporated into DNA only. Its minor role as mediator in cell wall metabolism (Ohkawa, 1979) may form a link, essential for survival, between the duplication of the only two macromolecules that can exist as single copies in the cell, namely DNA (nucleoid) and peptidoglycan (sacculus), but this link has yet to be deciphered.

The prokaryotic Cell Cycle Simulation (CCSim) program

The user-friendly Cell Cycle Simulation program (CCSim, http://simon.bio.uva.nl/cellcycle/) visualizes semi-realistically the relationships between the replication of the bacterial chromosome, cell mass-growth and division within the framework of the so-called Cooper–Helmstetter model (BCD dogma) (Helmstetter et al., 1968). CCSim exploits the four parameters τ, C, D and Mi to follow single cells during steady states of exponential growth and transitions such as nutritional shifts (Kjeldgaard et al., 1958) and changes in various other parameters that may be altered in mutants (Slater & Schaechter, 1974) or via experimental conditions that modify C (Pritchard & Zaritsky, 1970), D (Meacock & Pritchard, 1975) or Mi (Zaritsky & Zabrovitz, 1981; Wold et al., 1994). The default values of τ, C, D and Mi are 60, 40, 20 and 1 respectively, but they can easily be modified in steps of 1 or 10 min (and 0.1 for the last).

The main, default window of CCSim (Fig. 1) displays changes with time in cell mass (black line, M, in Mi units) (Hansen et al., 1991) rising exponentially, in DNA contents (magenta, G, in genome equivalent values) (Helmstetter et al., 1968) increasing linearly (right hand side scale), and the resultant, fluctuating DNA concentration (green, G/M) (Pritchard & Zaritsky, 1970), at a resolution of 1 min. Mass and DNA content change abruptly at the time of cell division, whereas the rates of change of DNA content and concentration (rise or drop, respectively) occur at the times of initiation and termination (Helmstetter et al., 1968), 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 clicking its side arrows) or automatically (by pressing the Run button once, or twice to stop), animated
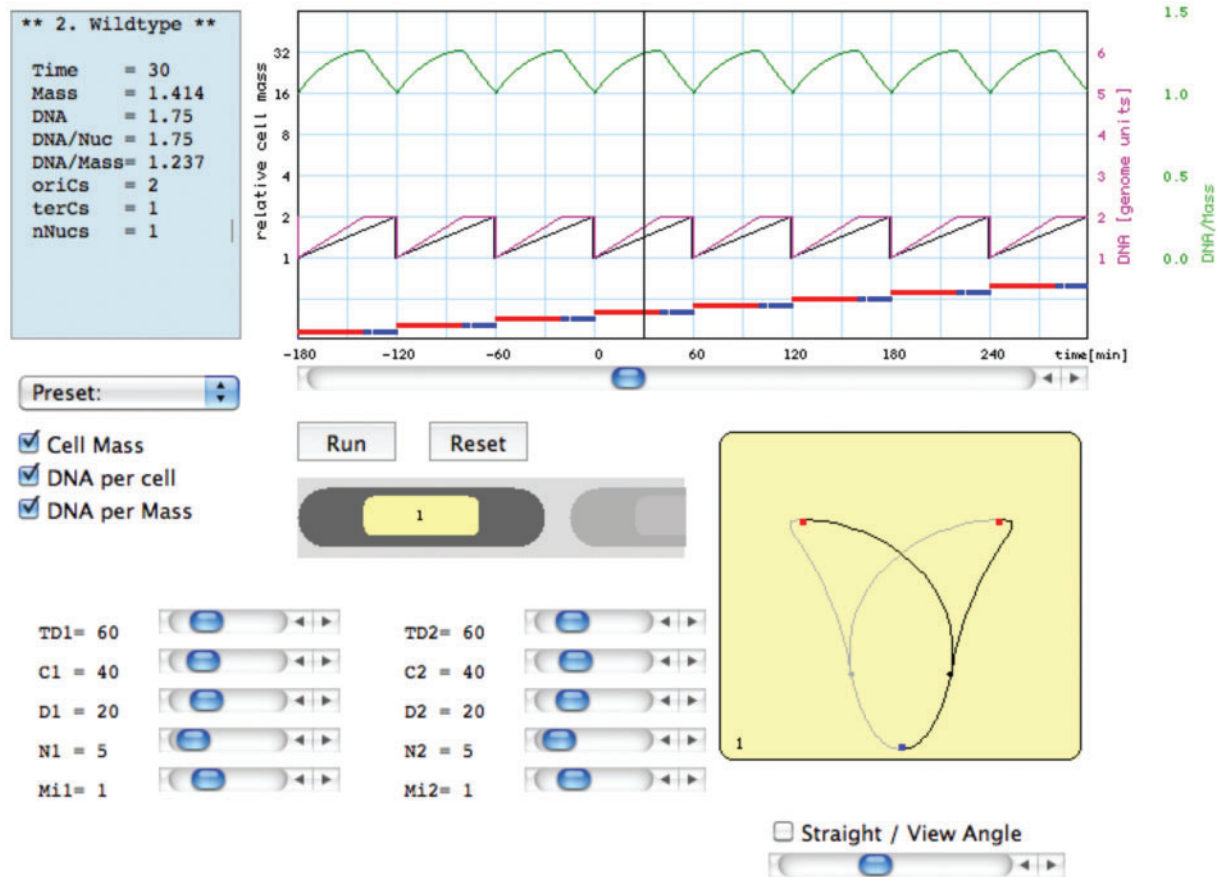
Fig. 1. The default view of CCSim as seen upon entrance to http://simon.bio.uva.nl/cellcycle/, explained and elaborated in the text. The program is freely available.

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 (Huls *et al.*, 1999). The black vertical line moves with time in correspondence with what is shown in two parallel windows of the screen that depict the following additional pieces of information:

• 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 optionally, linear half-chromosome) with *oriC*s (red dots), replisomes (black) and *terC*s (blue) at a 70° angle, with an optional change of the observation angle.

Other windows display the following:

• Links to a brief Explanation of the bacterial cell cycle, an extensive Glossary and options for Printing and Run Offline operations, displayed at the top.

• Two series of five scrollbars, the left set for pre-change parameters and the right set 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 both grow and constrict to two. The scheme lacks several features: the cell is not drawn in proportion to size because it can change mass over six octaves, it does not indicate the realistic change in cell width with growth rate, it does not follow exponential elongation with time, and it is concealed during transitions, while steady-state growth has not been reached; the missing items are not yet well defined.

• Five optional 'cases' are set automatically; the fifth includes an eclipse (E) – a minimal possible distance l_{min} between two successive replisomes (Zaritsky, 1975; Zaritsky *et al.*, 2007), as will be described below.

It is useful to study this setup by steps, from simpler conditions to increasingly complicated ones, as follows (Helmstetter *et al.*, 1968; Bleecken, 1969; Jiménez-Sanchez & Guzmán, 1988):
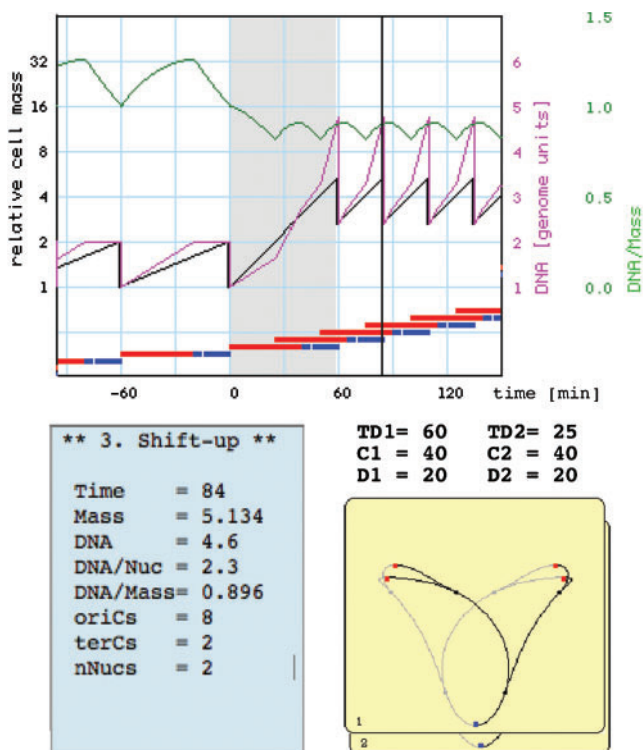
(a) $\tau > C + D$. Cell cycles do not overlap, i.e. B [$\equiv \tau - (C+D)$] is analogous to the eukaryotic *G1*, *C* to *S*, and *D* to *G2*. If $\tau = 70$ min for example, $B = 10$ min.

(b) $C + D > \tau > C$. That is $60 > \tau > 40$ min. Here, *B* disappears, and *Mi* is reached at the latter part of the mother's cell cycle, during the *D* period, to trigger an initiation event leading to the division of its daughter cell.

(c) $C > \tau > C/2$. Here, $40 > \tau > 20$ min, and initiation of replication occurs before the previous cycle has terminated, i.e. replication cycles overlap.

Fig. 2 depicts a cell 84 min after it was shifted up at birth ($t = 0$) from a relatively poor medium (with $\tau_1 = 60$ min) to a richer medium (yielding $\tau_2 = 25$ min), in which replication is initiated during the life cycle of the grandmother cell; this is 1 min before its first division under the new steady state (at $t = C + D + \tau$). In addition to the values of *M*, *G* and *G/M*, the configuration of the segregated daughter nucleoids is noteworthy: together, they include eight *oriC*s and six pairs of replisomes (three pairs upstream of each *terC*).

(d) $C/2 > \tau > C/3$, $C/3 > \tau > C/4$, etc. The degree of overlap becomes increasingly complicated and the number of replisomes rises exponentially with n ($= C/\tau$). Since $\tau_{min} = 20$ min, these conditions can only be achieved by extending *C* (e.g. by using thymine limitation) (Pritchard & Zaritsky, 1970; Ephrati-Elizur & Borenstein, 1971; Manor *et al.*, 1971). For $\tau = 40$ min, *C* would have to rise to above 80 min, 120 min, 160 min, etc. In general terms that means $C > modulus(\tau)$.

Fig. 3 depicts a *thyA* cell, growing with a doubling time $\tau = 40$ min, 109 min after it was stepped down at birth ($t = 0$) to a thymine concentration that slows chromosome replication rate by 2.25-fold (to $C_2 = 90$ min); this is 1 min before the last termination during the transition to the new steady-state (at $t = 130$ min). Here, and every 40 min henceforth, the cell contains eight copies of *oriC* and seven pairs of replisomes upstream of its single *terC*. One minute later (at 110 min), there would be two *terC*s per cell, each 'carrying' four *oriC*s and three replisomes (4/9th chromosome to be replicated for the following termination).

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

The eclipse concept and its consequences

The original, attractive notion that a bacterial chromosome can simultaneously entertain an unlimited number of replisomes (Sueoka & Yoshikawa, 1965) was challenged in early studies (Zaritsky, 1975). Recent results and insights support the thought that a limit to this number indeed exists (von Freiesleben *et al.*, 2000; Grigorian *et al.*, 2003; Simmons *et al.*, 2004; Nordman *et al.*, 2007; Zaritsky *et al.*, 2007; Rudolph *et al.*, 2009) – a limit that is probably due to lack of necessity: the time *C* taken to complete a round of replication in Thy^{+} cells never exceeds twice the minimal achievable doubling time (Helmstetter *et al.*, 1968). The maximum frequency of initiations is thus two per replication cycle and *E. coli* 'had no need' to evolve a system that can cope with more than two sets of successive replisomes operating simultaneously. This parallels the lack of an active uptake system for thymine (Reinhart & Copeland, 1973), which in Thy^{+} cells is not used as a DNA precursor directly (Pritchard, 1974). Consequently, mutants in *thyA* encoding inactive thymidylate synthase encounter an existential problem: thymine, used through a salvage pathway (Pritchard, 1974; Zaritsky *et al.*, 2006), enters the cell by diffusion, the pool of its metabolites is low and hence replication that depends on the external concentration is slower ('stepped-down') (Pritchard & Zaritsky, 1970; Ephrati-Elizur & Borenstein, 1971; Manor *et al.*, 1971). This, in turn, brings about the only known circumstance (when $C > 2\tau$) in which an exponentially grown cell needs more than two successive sets of replisome positions per chromosome operating simultaneously (Sueoka & Yoshikawa, 1965) to successfully compete with its neighbouring bacteria. Alas, it has not been prepared for this situation during the numerous generations of evolution!

The resolution of this existential need is by compromise between the number of replisomes necessary for duplication
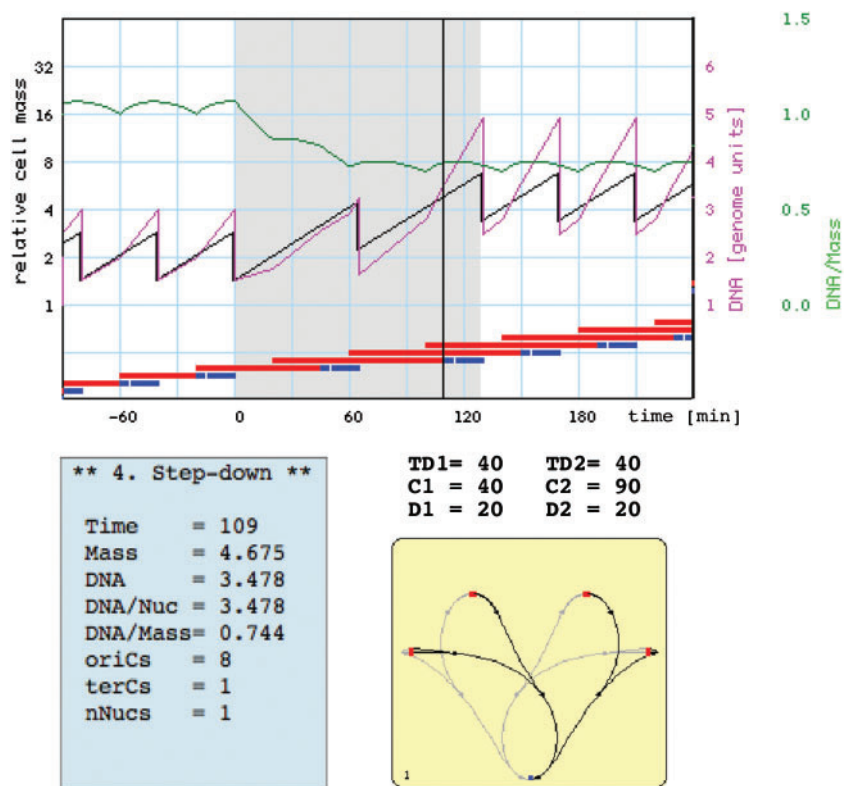

Fig. 2. An example of nutritional shift-up, generated by CCSim. A newborn cell in a culture growing exponentially under steady-state conditions is followed before and after a shift-up from $\tau_1 = 60$ min to $\tau_2 = 25$ min.

every τ min and the maximal possible distance between them, one that can be fatal: while the newly formed replisome attempts to proceed before its preceding one has reached a presumed minimal distance (so-called 'eclipse') from *oriC*, it will collide with its predecessor, resulting in double-strand DNA breaks (DSBs) at the newly made replisome, similar to those occurring during overexpression of *dnaA* (von Freiesleben *et al.*, 2000; Grigorian *et al.*, 2003; Simmons *et al.*, 2004; Nordman *et al.*, 2007; Felczak & Kaguni, 2009; Rudolph *et al.*, 2009). As long as the RecF system is active, these DSBs are repaired, allowing the 'stacked', collapsed replisome to resume replication when the preceding one is halfway between *oriC* and *terC* (i.e. has replicated one quarter of the length of the entire linearized chromosome). This procedure however takes time to achieve, and a 'deficit' in the number of actual initiations is built up as generations advance (Zaritsky *et al.*, 2007), while the capacity to initiate continues to accumulate in line with unaffected mass growth rate (Helmstetter *et al.*, 1968; Donachie, 1968; Pritchard *et al.*, 1969) (Fig. 4A). The delayed initiations are reflected by delayed divisions $(C+D)$ minutes later, normally occurring each mass doubling. The extra mass is accommodated by cells with increased diameters that can be ovoid (Zaritsky & Pritchard, 1973), spherical or monstrous (Zaritsky & Woldringh, 1978). Indeed (reviewed by Zaritsky *et al.*, 2006), thymine-limited cells cultivated in minimal salts-glucose (but not glycerol) medium grow indefinitely at a constant rate (with $\tau \approx 40$ min) but with delayed divisions in a mode defined

as 'normal' (Fishov *et al.*, 1995) – not steady-state because their size continuously increases.

Simulating a cell incurring eclipse: following initiation events by division frequency

The fifth optional case (e) in CCSim includes an eclipse – a minimal possible distance l_{min} between two successive replisomes (Zaritsky, 1975), which limits the number of replisones 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) (Zaritsky *et al.*, 2007), 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 replisome number is 3 [two so-called 'positions' n $(=C/\tau)$, calculated by 2^n-1] (Sueoka & Yoshikawa, 1965; Bleecken, 1969; Jiménez-Sanchez & Guzmán, 1988). Under such 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 $(L_{0.5}/l_{min})$ (Zaritsky *et al.*, 2007). Case (e) can thus explain the formation of such monstrous cells as seen in the Supplementary Video and the top left panel of Fig. 5 (time 0) during a long (7 h) evolution (Fig. 4A).
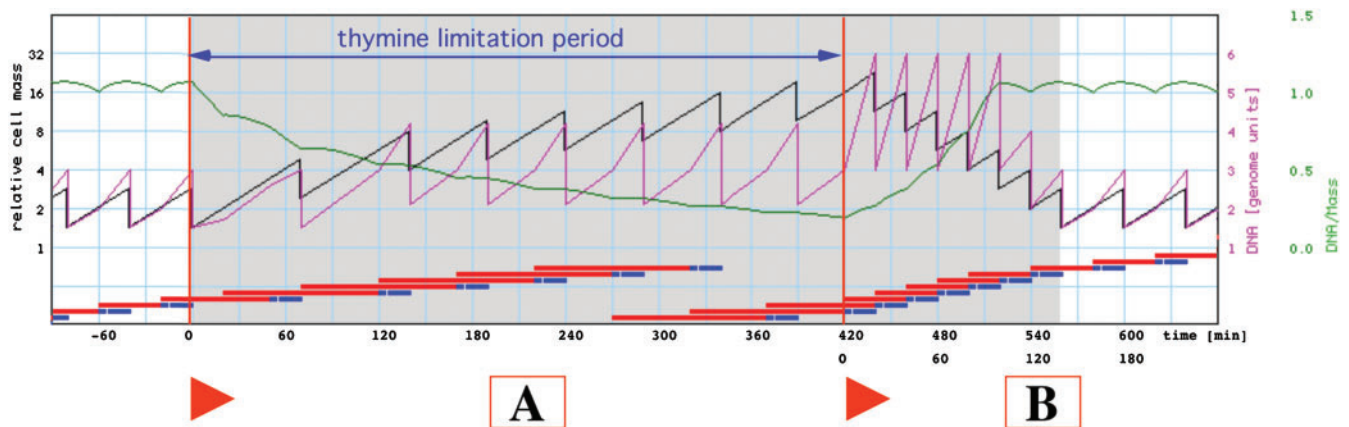
Fig. 4. Simulating a 'double-step' experiment under existence of an eclipse $E=0.5$, generated by CCSim. A glucose-grown ($\tau=40$ min) steady-state *E. coli* cell is stepped-down to long C ($=100$ min) conditions (A), and after 420 min (7 h), stepped-up back to conditions supporting a normal value of C ($=40$ min) (B). Red arrowheads and vertical lines indicate the times of transitions. Time 0 in B (restoration of thymine to high concentration) corresponds to 420 min in A.

According to this plausible, simplest explanation, the divisions 'deficit' will be restored as soon as the replisome movement is enhanced by stepping up to high thymine concentration, thus accelerating the rate of cell division. This notion can be followed in CCSim (Fig. 4B), and the prediction noted was tested here. Strain TAU-bar of *E. coli* 15T⁻ (*arg met pro trp ura thyA deoB*) (Hanawalt & Wax, 1964) was cultivated under severe thymine limitation (with $C \approx 100$ min) for a long period of time in M9-glucose medium. As before (reviewed by Zaritsky *et al.*, 2006, 2007), the thymine-limited cells showed a reduced rate of division without effects on mass growth rate. The consequent rise in cell size was accommodated by continuously enlarging width, then length, resulting in monstrous cells (e.g. Fig. 5, top left panel). Upon stepping up the thymine concentration and hence the rate of replication (Pritchard & Zaritsky, 1970), the changes that had occurred during the step-down (Fig. 4A) reversed as anticipated (Fig. 4B): the 'deficit' in cell divisions was restored (Fig. 5, Supplementary Video), reinstating the dimensions to near-normal after a sequence of more frequent divisions, consistent with fast approach of the replisomes to *terC* thus removing the 'veto' on division (e.g. Dix & Helmstetter, 1973). The unique phenomenon of 20 min intervals between at least five almost simultaneous, successive divisions forming some 32 cells from one during 100 min, while mass doubling time sustained at 40 min, is indeed predicted by CCSim (Fig. 4B).

Temporal coupling between replication and division

The time-lapse films and micrographs (e.g. Supplementary Video and Fig. 5) lend strong support to the eclipse concept (reviewed by Nordman *et al.*, 2007; Zaritsky *et al.*, 2007; Felczak & Kaguni, 2009). The enhanced frequency of divisions obtained by restoring the replication rate illustrates it as another aspect of the coupling between DNA replication and cell division but cannot simply be explained by the shorter C obtained at high thymine concentrations after step-up because this predicts enhancement of a single burst of divisions, as happens under slower growth rates when thymine is restored (e.g. Zaritsky & Pritchard, 1973), where $C<2\tau$. Under such circumstances, cell mass at initiation seems to remain constant and the mass accumulated during the longer $(C+D)$ period is accommodated by increased cell diameter without formation of aberrant cells.

When $C>2\tau$ (slow replication and fast growth rates), cells continuously increase their size (width, then length), culminating in monstrous shapes (Zaritsky & Woldringh, 1978; Zaritsky *et al.*, 2006). These 'monsters' perform multiple successive divisions at higher frequency during a subsequent step-up, reaching normal size and cylindrical shape (Fig. 5). This observation reflects another change in the cell cycle, and the simplest one envisaged with least additional assumptions is delayed initiations during the preceding thymine limitation, i.e. eclipse (Zaritsky, 1975; Zaritsky *et al.*, 2007). The question of its cause is moot: the description of 'collapsing replisomes' that cause DSBs, hence delaying the actual initiations (von Freiesleben *et al.*, 2000; Grigorian *et al.*, 2003; Simmons *et al.*, 2004; Nordman *et al.*, 2007; Rudolph *et al.*, 2009; Felczak & Kaguni, 2009), may seem unlikely (Conrad Woldringh, personal communication), given the structure and size of the compacted nucleoid and the fact that duplicated origins segregate immediately (Elmore *et al.*, 2005; Reyes-Lamothe *et al.*, 2008a, b). According to this view, when replication rate slows down and initiations are delayed in the eclipse mode, the processes that establish the orisomes or replication bubble [interplay between helicases, primases, DnaX-clamp-loaders and Pol IIIs that form the
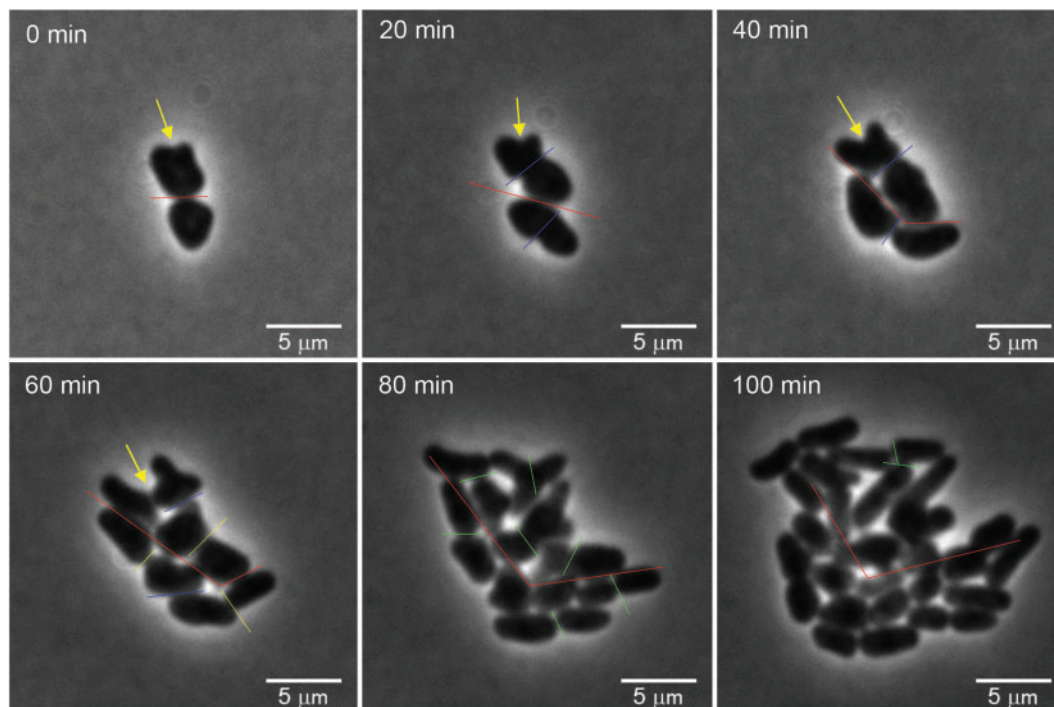
Fig. 5. Selected pictures (minutes, at top left corners of panels) from a time-lapse film (available as a Supplementary Video with the online version of this paper). Strain TAU-bar of *E. coli* 15T⁻ (Hanawalt & Wax, 1964) was cultivated in M9-salts medium with 0.4 % glucose as the sole carbon source, supplemented with the required amino acids (arginine, methionine, proline and tryptophan at 50 μg ml⁻¹ each), uracil (20 μg ml⁻¹) and thymine (1 μg ml⁻¹), with a doubling time of 41 ± 3 min. Mass growth was monitored as OD_{450} and sustained at an exponential, constant rate by successive dilutions into fresh, pre-warmed medium. Micrographs were taken at 2 min intervals starting 20–30 min after spreading the cells on agarose slides in a temperature-controlled chamber (37 °C) enveloping the microscope (Nikon Eclipse Ti fluorescence microscope equipped with a CCD camera, Photometrics Cool-Snap HQ2) at a ×100 magnification. The 1.5 % agarose contained the same medium as the batch culture, with 20 μg ml⁻¹ each of thymine and deoxyguanosine, imitating the wild-type rate of replication (Beacham *et al.*, 1971; Zaritsky *et al.*, 2006). The correct concentrations were achieved by mixing (1 : 1 v/v) the medium with agarose at double their final concentrations. The yellow arrows point to a 'split tip', i.e. a constricted pole of a large cell leading to a viable division at 60 min in a plane that is parallel to cell length (perpendicular to that of 'normal' divisions). Coloured lines indicate planes of synchronous divisions in hierarchical order: red, the first, forming two cells; blue, second set, forming four cells; yellow, third set, forming eight cells; green, fourth set, 'finally' losing synchrony (at 80 min; and see at 100 min and later in the film). The division planes are rotated relative to each other in 3D space (Zaritsky *et al.*, 1999; Zaritsky & Woldringh, 2003).

trombone-loop (Leonard & Grimwade, 2010)] may become hampered. However, the high synchrony of at least five successive divisions (Supplementary Video S1 and Fig. 5) exposes excess of building blocks for initiation and propagation of chromosome replication and for cell division that are accumulated during the step-down period independently of chromosome replication itself (Dix & Helmstetter, 1973; Jones & Donachie, 1973). In a batch, this synchrony is masked by a significant fraction of near-normal cells, cast off by the 'monsters' (Zaritsky & Woldrinph, 1978; Zaritsky *et al.*, 2006). We therefore believe that an initiating replisome 'collapses' into its predecessor, thus forming DSBs if the latter is too close (Zaritsky *et al.*, 2007), a view that has recently been confirmed and established by other means, biochemical, biophysical, genetic and physiological (von Freiesleben

et al., 2000; Grigorian *et al.*, 2003; Simmons *et al.*, 2004; Nordman *et al.*, 2007; Rudolph *et al.*, 2009; Felczak & Kaguni, 2009).

Spatial coupling between replication and division

Films (e.g. Supplementary Video S1) document *in vivo* and *in situ* that cell poles are not always inert and confirm that old poles can, under certain circumstances, synthesize peptidoglycan (de Pedro *et al.*, 2004): wide cells can constrict and split to two daughters in a plane perpendicular to the previous division plane, i.e. parallel to their length axis (e.g. yellow arrows in Fig. 5, panels 0–60 min). Several models exist for the physical mechanisms by which FtsZ assembles and generates the force to constrict (described and discussed by Erickson, 2009). An attractive

biological explanation relates the positioning of the site of constriction by FtsZ to that between the segregating nucleoid during replication through the so-called 'transertion strings' – standing for the coupled *trans*cription, *trans*lation and in*sertion* of proteins into and through the membrane (Norris, 1995; Woldringh *et al.*, 1995; Zaritsky *et al.*, 1999; Woldringh, 2002; Zaritsky & Woldringh, 2003; Rabinovitch *et al.*, 2003). Spherical cells containing many nucleoids (Zaritsky *et al.*, 1999; Zaritsky & Woldringh, 2003) divide in perpendicular planes and in between segregating nucleoids. Is there a link between the segregation and surface curvature (Huang *et al.*, 2006) to signal the site for FtsZ ring assembly? How does the so-called nucleoid 'complexity', defined as the number of replication positions (Zaritsky *et al.*, 2006), affect cell diameter? Some progress was recently achieved in deciphering the mechanism governing 'nucleoid occlusion' in *E. coli* (Bernhardt & de Boer, 2005; Cho *et al.*, 2011; Tonthat *et al.*, 2011). Whether or not the bacterial division mechanism is analogous to that in eukaryotic cells remains to be seen, and the apparent spatial coupling between segregating nucleoid and division site is yet to be unequivocally determined.

Remarks about the CCSim program and beyond

Several additional remarks are noteworthy and instructive.

● In steady-state, exponentially growing cultures (Fishov *et al.*, 1995), both mean cell mass M ($=\ln 2\, Mi\, 2^{(C+D)/\tau}$) and DNA content G ($=\tau[(2^{(C+D)/\tau}-2^{D/\tau})/C \ln 2]$) rise with growth rate (inverse of τ), whereas DNA concentration G/M ($=\tau(1-2^{-C/\tau})/Mi\, C \ln 2$) decreases because the former rises faster. The same happens at slower replication rates (extended C). It has been demonstrated that the usual amount or concentration of DNA (G or G/M, respectively) is not limiting for normal cell growth (Schaechter *et al.*, 1958; Maaløe & Kjeldgaard, 1966; Pritchard & Zaritsky, 1970; Zaritsky & Pritchard, 1973; Zaritsky & Woldringh, 1978; Zaritsky *et al.*, 2006), but the lowest limit of G/M required for a cell to survive and multiply indefinitely must still be determined.

● 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 rates at which a cell breaching the eclipse changes its DNA concentration, size and dimensions depend on the degree to which the eclipse is breached, i.e. by what factor the actual number of positions is smaller than that needed to maintain steady-state growth. Thus, increasing the growth rate or decreasing the replication rate similarly enhance formation of highly irregular cells, as was also observed in the 1960s for fast-growing *thyA* mutants (Chai & Lark, 1970).

● The meaning of D is yet to be explained in molecular terms; it was empirically defined as the difference between ($C+D$) and C, and the existing, feasible explanation to its constant value (Zaritsky *et al.*, 2007) must still be substantiated. The rule of constant D seems to break under thymine limitation (Zaritsky & Pritchard, 1973; Meacock & Pritchard, 1975), but without knowing how it changes with C, it is usually kept constant.

● Other parameters that must be taken into account when considering whole populations (not needed for the current CCSim program) are the degrees of variation, so-called 'noise', in doubling time (Powell, 1956), size (Koch & Schaechter, 1962) and division symmetry (Trueba & Woldringh, 1980) among the individual cells. These should be dealt with when CCSim is extended.

Concluding remarks

This review summarizes the basics of bacterial growth, chromosome replication and cell division, and presents a combination of powerful tools to study the bacterial cell cycle, to develop hypotheses with experimentally testable predictions, and to analyse the results obtained. The CCSim program accommodates a large number of options that cannot always be intuitively visualized, enabling the testing and verification of an idea raised 35 years ago (Zaritsky, 1975). It is a powerful tool for testing the behaviour of the large number of mutants currently available with cell-cycle alterations (e.g. Leonard & Grimwade, 2010). The highly specific and simple mode by which the rate of DNA replication is manipulated yields a method that avoids pleiotropic effects caused by changing medium composition, drugs or temperature shifts. CCSim is useful for students at the graduate and undergraduate levels (personal experience of A. Z.) as well as to scientists who investigate aspects of the bacterial cell cycle (Zaritsky *et al.*, 2006, 2007). It must however be borne in mind that the picture in real life is not as idyllic as depicted in CCSim.

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