Bacterial Growth and Division

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Keywords

C-period
The time for a round of DNA replication, from initiation at the origin to completion at the terminus. In *Escherichia coli*, the C-period, for cells growing between 20- and 60-min doubling times, is a constant of approximately 40 min.

D-period
The time between termination of DNA replication and cell division. In *Escherichia coli*, the D-period, for cells growing between 20- and 60-min doubling times, is a constant of approximately 20 min.

Multiple Forks
The situation that occurs when initiation of DNA replication occurs on a molecule that has not finished its previous round of replication; there are forks upon forked material.

Cytoplasm
The collective name for the numerous components of the cell that are not associated with either the cell wall or the genome. It is composed of the small molecules such as ions, metabolites, and cofactors, macromolecules such as soluble enzymes, tRNAs, mRNAs, and higher complexes such as ribosomes.

Peptidoglycan
The stress-bearing, presumably shape-maintaining, layer of the bacterial cell wall. It is composed of glycan chains cross-linked with amino acids.

Cell Membrane
The lipid-containing structures that lie adjacent to the peptidoglycan layer. Some cells have a single membrane layer, while others have two different membranes, an outer and an inner membrane.

Segregation
The distribution of cell material from the dividing mother cell into the two daughter cells. Different cell components may have different segregation patterns.

Origin
A sequence on a chromosome or a plasmid where normal replication of DNA initiates.

Terminus
A point or region on a chromosome or plasmid where a round of replication terminates.

Bidirectional Replication
Replication of DNA with two replication forks proceeding in opposite directions away from the origin of replication.

GC-skew
The excess (or deficit) of G residues over C residues within a single strand of DNA. While $G = C$ in a double helix, an individual strand may have an excess of G within a region or an excess of C residues within a region.

FtsZ Ring
The ring of synthesis of the pole region during pole formation.

■ A dividing bacterial cell must, on average, have precisely twice as much of everything found in a newborn cell. How does a bacterial cell ensure that all cell components are duplicated between divisions, and how does a cell ensure that it does not divide prior to duplicating all of its components? These questions have been investigated by studying biosynthesis of cell components during the division cycle. Cell cytoplasm increases uniformly and exponentially during the division cycle. There does not appear to be any cell-cycle-specific syntheses of cytoplasmic components during the division cycle of a well-studied bacterium such as *E. coli*. DNA replication is initiated at a specific time in the division cycle when the cell mass per chromosome origin reaches a particular value. Once initiated, DNA replicates at a constant rate such that the time between initiation and termination of replication is relatively independent of the growth rate. The time between termination of replication and cell division is also relatively constant. High-copy plasmids replicate throughout the division cycle and low-copy plasmids replicate at a particular time during the division cycle according to rules similar to that for initiation of chromosome replication. The cell surface grows in response to the increase in cell mass so that the turgor pressure inside the cell, and the cell density, are constant during the division cycle. At division, the components of the cell are segregated to the two daughter cells. The cytoplasm segregates randomly, the DNA segregates stochastically but nonrandomly, and the peptidoglycan segregates in a manner consistent with the fixed location of the synthesized peptidoglycan. The key "events" during the division cycle are the initiation and termination of DNA replication and the initiation and termination of pole formation. There do not appear to be other cell-cycle-specific events or syntheses during the division cycle that are related to the regulation of the division cycle.

1
Growth of Bacterial Cultures

1.1
The Classical Growth Curve

For almost a century (since Buchanan, 1918), the classic growth curve has been used as a description of the pattern of bacterial growth. An overgrown culture is diluted into fresh medium and at first there is a "lag" phase, experimentally defined as a period before viable cell number begins to increase. Then cell numbers begin to increase and the culture enters the "log" phase. The log phase is the period of exponential increase in cell number. Although the cells during this period of steady state cell number increase are growing exponentially, the cells are generally referred to as being in "log" phase because of the usual plotting of cell number on semilogarithmic coordinates. During the log phase, plotting cell number increase on semilogarithmic paper yields a straight line that can be used to determine the doubling time of a culture. After the cells grow to higher concentrations, cell growth slows as the cells enter the stationary phase. Cell growth then ceases. Finally, if a culture is studied long enough, cells begin to lose viability or the ability to form colonies, and the culture enters the death phase.

During these phases, there are changes in cell sizes. The initial cells in the overgrown culture are small, cell size increases during the lag phase, cells are largest during the log phase, and then cell size decreases as cells enter the stationary phase. This classical view of the way in which cells grow is really a special case of the phenomenon of changes in cell size with changes in growth rate.

1.2
Bacterial Growth at Different Growth Rates

It is possible to grow a given bacterium in cultures at a wide range of growth rates. For example, it is possible to grow *E. coli* with a 300-min interdivision time with serine as the sole source of carbon, and a little faster than 20-min interdivision times in various complex broths available for bacterial culture. By varying the medium composition, it appears that any possible growth rate is allowable, so that there is a continuous range of allowable growth rates between the extremely slow and the extremely fast.

When bacteria are grown at a given temperature in different media at a number of different growth rates, a regular pattern of cell composition and cell size is observed. The faster a cell grows (richer medium) the larger the cell and the more the DNA, RNA, protein, and other components per cell (Fig. 1). When cell components are accurately measured, it is found that the material per cell increases exponentially as a function of the reciprocal of the doubling time (i.e.

growth rate, as a shorter doubling time indicates a faster growth rate).

1.3
The Shift-up

During a shift-up from a slow growth medium (e.g. minimal medium, in which cells are small) to rapid growth in a rich medium (e.g. nutrient broth, in which cells are larger), there is a regular pattern of change in cell size and composition. Immediately after the shift-up, there is a rapid change in the rate of RNA and protein synthesis to the new rate of increase. There is a slower change in the rate of DNA synthesis. There is a delay before there is any change in the rate of cell number increase. Because of
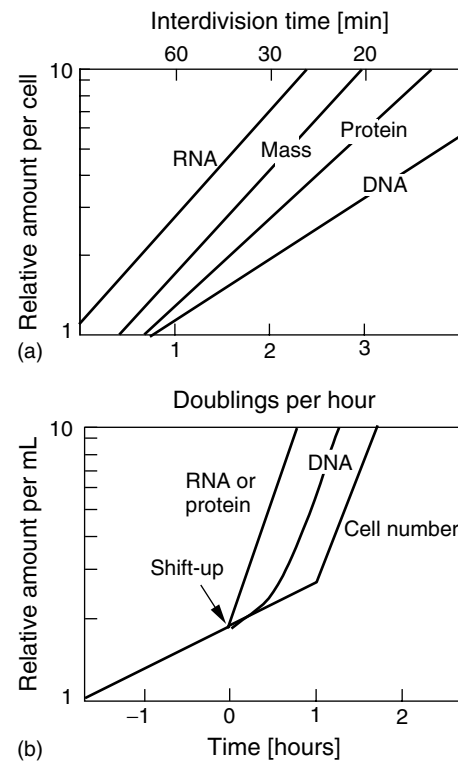

Fig. 1 (a) Composition of bacteria as a function of growth rate. (b) Synthesis of cell components during a shift-up from minimal medium to faster growth in richer medium.

the dissociation of mass increase and cell number increase, the average cell size increases during this first part of the shift-up. After a period of time, there is a sharp change in the rate of cell increase from the old rate to the new rate. This occurs 60 min after the shift-up (Fig. 1). Because there is a lag in the change in the rate of cell division, while cytoplasm synthesis increases to the new rate almost immediately, during the shift-up period there is an increase in cell size producing the larger-sized cells in the faster growth medium.

1.4
Classical Growth Pattern Is Merely a Series of Shift-ups and Shift-downs

The classical growth pattern for a culture can now be seen as a series of shift-ups and shift-downs. When an overgrown culture (nongrowing, and thus of essentially zero growth rate) containing small cells is diluted into fresh medium, there is a "shift-up" in growth rate. The mass increases immediately with no cell division occurring. The "lag" phase is thus one of a lag in cell number increase, but there is essentially no lag when considering mass increase. After the normal cell size is achieved for that medium, both the mass and the cell number increase in parallel. The rate of cell increase is determined by the medium supporting the more rapid growth. There is a constant larger-size cell during exponential growth. The reverse occurs as growth begins to slow down and the cells approach the stationary phase. The rate of mass synthesis decreases first, followed later by a cessation in the increase in cell number. Because division continues after mass increase ceases, the average cell size decreases in an overgrown culture.

2
The Bacterial Division Cycle

2.1
Cell Age and the Age Distribution during Balanced Growth

By convention, a newborn cell has an age of 0.0 and grows during the division cycle to divide at age 1.0. The cell ages during the division cycle are thus numbers between 0.0 and 1.0 indicating where, in the division cycle, a cell is located at a particular instant. A cell half-way between birth and division is age 0.5. Cell age, by definition, increases linearly during the division cycle. However, cells at the same absolute time after birth are only approximately the same cell-cycle age because of the variability in the absolute time required for the division cycle of an individual cell.

In a growing culture, the distribution of cell ages is not uniform. There are twice as many newborn cells as dividing cells. The age distribution is given by the formula $N = 2^{1-x}$, where x is the cell age between 0.0 and 1.0. This formula indicates that there are twice as many newborn cells ($x = 0.0$) as dividing cells ($x = 1.0$). If all cells grew with exactly the same interdivision time, this formula would give the age distribution exactly. However, because of cycle variability there is a smoothing of the function and the actual distribution is an approximation of the theoretical distribution. Because of the age distribution, the properties of an exponentially growing culture are independent of time. This means that all patterns of synthesis within the division cycle give an exponentially increasing amount of material in a growing culture. This is because the age distribution is constant during steady state, exponential growth. Thus, whether something is

made linearly, exponentially, or at an instant during the division cycle, in an exponentially growing culture the amounts of all cell components increase exponentially and in parallel. This is referred to as balanced growth. The observed balanced growth is the result of the age distribution during growth being invariant.

Given that between the birth of a cell and its subsequent division, all of the cell components double, we can ask, "At what rate is material synthesized during the division cycle of an individual cell, and how does the cell ensure that there is a precise doubling of cell components by the time the cell divides?" Let us first analyze which components must be dealt with in terms of the division cycle.

2.2
Aggregation Theory and the Control of Synthesis during the Division Cycle

In economics, the aggregation problem is how to combine various sectors of an economy. Should the figures for the production of capital machinery be combined with those for the production of consumer goods? Is paper produced for boxes in the same economic category as stationery? It is difficult to treat each item in an economy individually; some aggregation is necessary in order to understand the whole system.

Now, consider the aggregation problem for the analysis of the bacterial division cycle. How should one aggregate the different cell components in order to achieve an understanding of the biochemistry of growth and division? Is there a unique pattern of synthesis during the division cycle for each enzyme, or are there a limited number of patterns with different enzymes or molecules synthesized according to any

one of these patterns? Are there ways of grouping proteins or RNA molecules so that one can consider classes of molecules rather than individual molecular species? Should we consider the cell membrane as a different category from that of peptidoglycan? There are approximately a thousand proteins in the growing cell, and if each protein had a unique cell-cycle synthetic pattern, or if there were only a few enzymes exhibiting any particular pattern, we would have an insuperable task describing the biosynthesis of the cell during the division cycle.

Fortunately, one need consider only three categories of molecules, each of which is synthesized with a unique pattern. The growth pattern of the cell is the sum of these three biosynthetic patterns. The first category is the cytoplasm, which is the entire accumulation of proteins, RNA molecules, ribosomes, small molecules, water, and ions that make up the bulk of the bacterial cell. It is the material enclosed within the cell surface that is not the genome. The second category is the genome, the one-dimensional linear DNA structure. The third category is the cell surface, which encloses the cytoplasm and the genome. The surface is composed of peptidoglycan, membranes, and membrane-associated proteins and polysaccharides. Everything in the cell fits into one of the three categories, and each category has a different pattern of synthesis during the division cycle. These three patterns are simple to understand as they can be derived from our current knowledge of the principles involved in the biosynthesis of the cytoplasm, the genome, and the cell surface.

The division cycle of bacteria in general may be best described by discussing the division cycle of the bacterium about which most is known, *E. coli*, and then describing

other bacteria and their patterns of growth and division with respect to this archetypal pattern. The division cycle of *E. coli* will be described, indicating how the various components of the cell are made and distributed into two new daughter cells.

3
Synthesis of Cell Components during the Division Cycle

3.1
Cytoplasm Synthesis during the Division Cycle

3.1.1 Exponential Cytoplasm Synthesis during the Division Cycle

Consider a unit volume of cytoplasm. It contains enzymes required for the breakdown of nutrients, enzymes for the production of energy for biosynthesis, enzymes for the biosynthesis of low molecular weight precursors of macromolecules, and enzymes for the synthesis of macromolecules. Each unit of cytoplasm produces a small amount of new cytoplasm over a small interval of time. If the new cytoplasm is indistinguishable from the old, and if the new cytoplasm acts to synthesize more cytoplasm immediately, then the pattern of cytoplasm synthesis is exponential. This is because after each interval of synthesis, the rate of synthesis increases owing to the new increment of material added to the cytoplasm. This exponential pattern is illustrated in Fig. 2 with both the rate of synthesis and the amount of material increasing exponentially during the division cycle.

Fig. 2 Cytoplasm synthesis during the division cycle. Both the rate of cytoplasm synthesis and the pattern of accumulation of cytoplasm are exponential during the division cycle.

The pattern for cytoplasm as a whole is reflected in the synthesis of the individual cytoplasmic components. There are no changes in the specific rate of synthesis or pattern of cytoplasm synthesis that are related to any particular cell-cycle event. (Actually, there is a minor *caveat* to this generalization, which is treated below.) No particular molecule of the cytoplasm is made differently from any other molecule of the cytoplasm during the division cycle. In addition, there is no variation, during the division cycle, in the relative concentration of any component of the cytoplasm. All parts of the cytoplasm accumulate exponentially during the division cycle, and this pattern is unchanged even when the cell is dividing. At the instant of division, the combined rate of cytoplasm synthesis in the two new daughter cells is precisely equal to the synthetic rate in the dividing mother cell.

One minor exception to the rule that specific proteins are made exponentially during the division cycle regards the effect of momentary sequestration of DNA upon replication. Specific regions of DNA are sequestered in the membrane at different times during the cell cycle at the time of their replication. During this sequestration period, the DNA is not available for transcription. Thus, for all genes there

Rate of cytoplasm synthesis [log scale]

Amount of cytoplasm per cell [log scale]

Cell age
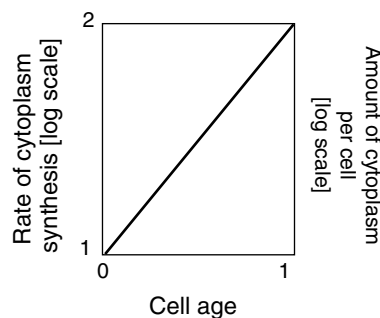
is a time during the division cycle at which gene expression is prohibited. This momentary cessation of transcription should not obscure the more fundamental point that there does not appear to be any specific control of gene expression for any gene during the division cycle that is related to the regulation of that gene during the division cycle. One should imagine this cessation of gene expression as merely a necessary accommodation of transcription to the needs of the cell for DNA replication. It could be imagined that if the cell were able to replicate DNA without any cessation of transcription, there would be no reason to expect that the cell would alter specific gene expression at any point during the division cycle.

The evidence for a smooth, continuous, and exponential increase in cytoplasm comes from experiments that use the membrane-elution method, colloquially referred to as the *baby machine* (see below). The pattern of DNA synthesis during the division cycle, which was subsequently shown to be correct by flow-cytometric and other analyses, was actually discovered using the membrane-elution method. Thus, the results from a method capable of producing an accurate analysis of biosynthesis during the division cycle have confirmed the exponential synthesis of cytoplasm.

In addition to this experimental support, an evolutionary argument can be made for an invariant rate of accumulation of cytoplasm during the division cycle. If the synthetic rate of an enzyme changed abruptly during the division cycle, there would be a relative excess or deficiency of that enzyme at some point during the division cycle; this would be an inefficient use of resources. The optimal allocation of resources is for each component to be at a constant concentration during the division cycle. Cells would not evolve controls that make them less efficient in cell production. The ideal pattern of cytoplasm synthesis is an invariant cytoplasm composition during the division cycle.

A third argument supporting exponential cytoplasm synthesis is that only this pattern is explained in known biochemical terms. The cycle-independent exponential synthesis of cytoplasm can be derived from our current understanding of the biochemistry of macromolecule synthesis. Enzymes make RNA and protein, which make ribosomes, which then lead to more protein synthesis. More proteins mean more RNA polymerases, more catabolic and anabolic enzymes, and the continuously increasing ability of the cell to make more and more cytoplasm. In contrast, if newly synthesized material were *not* active or available for synthesis when made, but instead was recruited for biosynthesis only at the instant of division or at some specific cell-cycle age, one would have linear cytoplasm synthesis during the division cycle. There are no known mechanisms enabling the cell to distinguish between newly synthesized cytoplasm and old cytoplasm, or which lead to cell-cycle-specific variation in the synthetic rate of any particular molecule during the division cycle. We therefore conclude that in theory as well as in practice, cytoplasm increases uneventfully and exponentially during the division cycle.

3.1.2 Variation in the Rate of Cytoplasm Increase

The continuous variation in allowable growth rates may be understood by partitioning the cytoplasm into those molecules involved in cytoplasm synthesis and that are absolutely necessary for growth (ribosomes, RNA polymerases, etc.) and those molecules that are involved in dispensable

syntheses (e.g. enzymes involved in amino acid biosynthesis). In a minimal medium, in which many dispensable enzymes are being synthesized, the ribosomes may be considered to be involved in the synthesis of ribosomal protein and a large number of dispensable proteins. Since only a fraction of the ribosomes are making ribosomal protein at any moment, the time for a doubling in the ribosomal content is relatively long. As one increases the number of nutrients in the medium, thus relieving the cell of having to make the dispensable enzymes, a larger fraction of ribosomes are involved in ribosomal protein synthesis. Thus, in rich medium the time for ribosome number to double is shorter than in poor medium. The variation in growth rates is thus a reflection of the continuous ability of the cell to partition the ribosomes (or the RNA polymerases) between making ribosomal components or nonribosomal components.

Considering the time required for a ribosome to make another ribosome (i.e. using 15 amino acids incorporated in a protein chain in a second), one can calculate that it would take eight minutes for one ribosome to synthesize a single ribosome. If a cell were in some way supplied with all proteins other than ribosomes, one could consider that the fastest rate of cell doubling would be eight minutes, the time for ribosomes to double their number.

3.2
DNA Synthesis during the Division Cycle

One of the most important generalizations regarding the regulation of linear macromolecule synthesis is that the rate of synthesis is regulated at the point of initiation of polymerization. This principle is exhibited most clearly in DNA replication, where the process of initiation of replication can be clearly dissociated from the continued replication of DNA that has already initiated replication. If one inhibits the initiation of replication, then the rounds of DNA replication in progress will continue until their normal termination.

3.2.1 Pattern of DNA Synthesis during the Division Cycle

The DNA synthesis pattern during the bacterial division cycle (specifically for *E. coli* and related bacteria) is composed of one or more periods of constant rates of DNA accumulation (Fig. 3). In cells with a 60-min interdivision time, there is a constant rate of DNA synthesis for the first 40 min, with one bidirectional pair of replication forks, followed by a zero rate of synthesis during the last 20 min. In cells with a 30-min interdivision time, there is a constant rate of DNA synthesis for the first 10 min, which falls to a constant rate that is two-thirds of the initial rate for the last 20 min. In cells with a 20-min interdivision time, there is a constant rate of DNA synthesis throughout the division cycle. Other growth rates have similar particular patterns of DNA synthesis.

The constant rates of DNA synthesis can be understood in terms of the molecular aspects of DNA synthesis. DNA is synthesized by the movement of a replication point along the parental double helix, leaving two double helices in its wake. The rate of movement of a growing point appears to be invariant and independent of its location in the genome. This means that the rate of DNA synthesis is proportional to the extant number of replication points. The number of replication points is constant for any period of the division cycle. Thus, the rate of DNA synthesis during any period of the division cycle is also constant. The
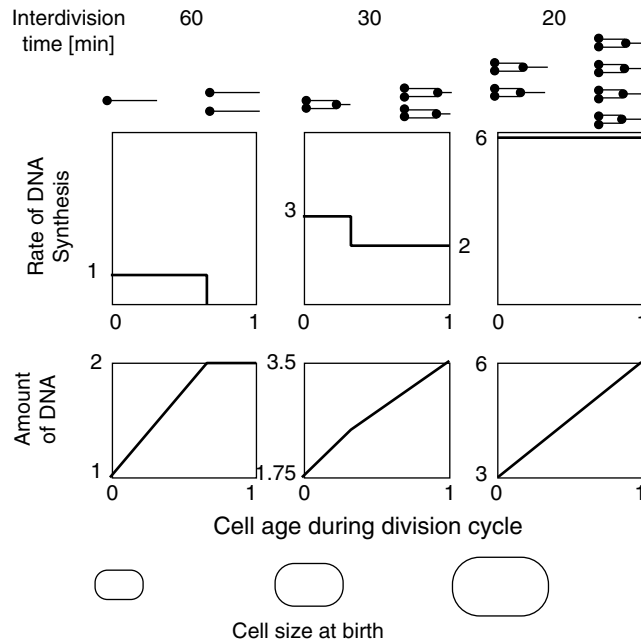
Fig. 3 DNA synthesis during the division cycle. Three different patterns are illustrated for cells growing with 20, 30, and 60 min doubling times. For each growth rate, the time from initiation to termination of DNA replication is 40 min. The time between termination of replication and cell division is 20 min. The proposed chromosome patterns at the start and finish of the division cycle are illustrated above the graphs. In addition to the rate of DNA synthesis during the division cycle, the pattern of accumulation of DNA during the division cycle is presented. Accumulation of DNA is composed of periods of linear synthesis. The rates during these periods are proportional to the existing number of growing points. The graph of the rate of synthesis is the differential of the accumulation plot. A representation of the cell size expected for cells at the start of the division cycle is presented below each synthetic pattern. The size of the newborn cells is in the ratio of 1 : 2 : 4 in the three cultures illustrated here. The number of new initiations at the start of each division cycle is also in the ratio of 1 : 2 : 4.

pattern of constant rates of synthesis is derived from, and is consistent with, our understanding of the biochemistry of DNA synthesis.

Embedded in the patterns of Fig. 3 are the two major rules for the determination of the pattern of replication of DNA during the bacterial division cycle. The first rule is that once DNA replication is initiated at the origin of replication, the time for a complete round of replication – the C-period – is relatively invariant (40 min in *E. coli* at 37 °C). The second rule is that the time between termination of replication and cell division – the D-period – is also relatively invariant.

The constant C- and D-periods are most clearly evident in the 60-min cells in Fig. 3.

At other growth rates, the patterns of synthesis appear more complicated, but this is due to the possibility of initiation occurring before completion of previous rounds of replication and the possibility of initiation of replication occurring in one division cycle and replication of the chromosome being completed in a subsequent division cycle.

3.2.2 Regulation of Initiation of DNA Replication

Why is replication initiated at these particular times during the division cycle? How is the initiation process regulated? Rounds of replication are initiated in a bacterial cell when the amount of cell mass or something proportional to the cell mass (the ''initiator'') is present at a certain amount per origin. Initiation of new rounds of replication occurs once per division cycle when the mass (or ''initiator'') per origin reaches a fixed value. In the 60-min cell, initiation occurs at one origin with a cell of unit size (size 1.0). In the 30-min cells, there are two origins in the newborn cell and the size of the newborn cell is twice that of a 60-min cell. And the 20-min cell has four origins initiated in the newborn cell and the cell size is four times the newborn 60-min cell. Thus, not only does the DNA per cell increase with growth rate but the average cell size increases with growth rate as well. In the cells shown in Fig. 3, the relative sizes of the newborn cells, as well as the average cell in the culture, are in the ratio of $1:2:4$. This increase in cell size with growth rate is consistent with the exponential increase in cell size as a function of the inverse of the doubling time.

The actual nature of the initiator is not known, but a number of candidate molecules have been identified. Among these, the DnaA protein appears as a good candidate for the regulator of DNA initiation.

Alternative models of initiation have been proposed, such as the sudden accumulation of an inhibitor of initiation that is diluted out by cell growth. This model does not accommodate the constant synthesis of cell-cytoplasmic components. In all cases, it is indistinguishable from the initiator accumulation model, and there is no evidence for such an inhibitor being synthesized during the division cycle.

3.2.3 The Specific Origin of Replication

A specific sequence of DNA in the bacterial genome is the origin of replication (oriC). The required sequence (for *E. coli*) is 245 bp. Within this sequence are 5 highly conserved sequence blocks of 15 to 20 bp. These conserved regions are interspersed with sequences that are random. Regions of random sequence but of fixed length separate the conserved regions. Thus, the length of separation of conserved sequences is more important than the specific sequence between the conserved sequences. Four sequences of 9 bp are specific recognition sites for the binding of dnaA, the specific initiator protein. In addition, there are three 13-bp regions that are sites for opening the duplex DNA for initiation. Finally, there are 14 GATC sequences located in the origin (oriC) region and the neighboring regions. These 14 four-bp sequences are expected to occur at a much lower frequency if they were not a necessary part of the *E. coli* origin.

The ultimate proof that this sequence is the origin of replication is the production of minichromosomes, small replicating circles of DNA that have the bacterial origin of replication. An *E. coli* cell can have many copies of the minichromosome

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without any apparent impairment of cell growth.

3.2.4 Protein Factors Controlling DNA Replication

Initiation of replication of DNA is a function of numerous proteins. The key regulatory protein appears to be the product of the *dnaA* gene. The dnaA protein binds to the 9-bp "dnaA boxes" in the oriC region. After binding to the origin, the protein promotes melting of the DNA (i.e. separation of the DNA strands in a local region) and helps in loading additional proteins such as the dnaB protein. After this initial opening of the DNA strands, replication fork movement is allowed to proceed bidirectionally from the origin.

Initiation of replication is distinct from the continued replication of DNA that has been initiated. Once initiated, DNA replication can continue even though the accumulation of cell cytoplasm is inhibited.

3.2.5 Chromosome Organization and Bidirectional Replication

The phenomenon of bidirectional replication has two effects on the organization of the chromosome. One effect is the variation in the relative abundance of G residues versus C residues in one of the strands. While G = C in the double helix, such an equality need not exist in a given strand or in a particular region of a strand. As a general rule, it is found that in the two halves of the bidirectionally replicated bacterial genome, one half of the chromosome (e.g. that from noon past three down to six on a clock representation of the genome) has G > C and the other half (noon, past nine, down to six) has G < C. If one calculates the $G - C/G + C$ for a running window of nucleotides, and accumulates the value

of this "GC-skew," there is a valley that corresponds to the origin and a peak that corresponds to the terminus. As long as one reads the strand in the 5′ to 3′ direction, the location of the peak and the valley is independent of the strand being used to define the origin and the terminus.

The bidirectional organization of the genome also affects the direction of gene transcription. As a general rule (with numerous exceptions), the direction of gene transcription is in the same direction as the movement of the replication fork. The rationale for this phenomenon is that this correspondence of direction of replication and transcription reduces the "head-on collision" of replication and transcription. The fact that there are many exceptions means that the replication system can accommodate running into transcriptional units without a problem.

3.2.6 DnaA Protein Is Involved in Initiation of DNA Replication

The DnaA protein, identified by mutants affecting the initiation of DNA replication and not the progression of rounds of replication already in process, appears to be the ultimate regulatory protein with regard to the initiation of DNA synthesis. The initiation process involves the cooperative binding of approximately 20 DnaA protein molecules to the origin of replication. This complex then leads to the open complex in which a region of AT-rich 13-mer repeats denatures so that single-stranded DNA is exposed. Other proteins such as the DnaB helicase are now introduced into the forks of the melted DNA where it expands the region of single-stranded DNA. Other molecules then join the initiation complex with the final loading of DNA polymerase III. When this occurs, the replication of DNA progresses

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around the genome to the opposite end of the genome where replication terminates.

3.3
Surface Synthesis during the Division Cycle

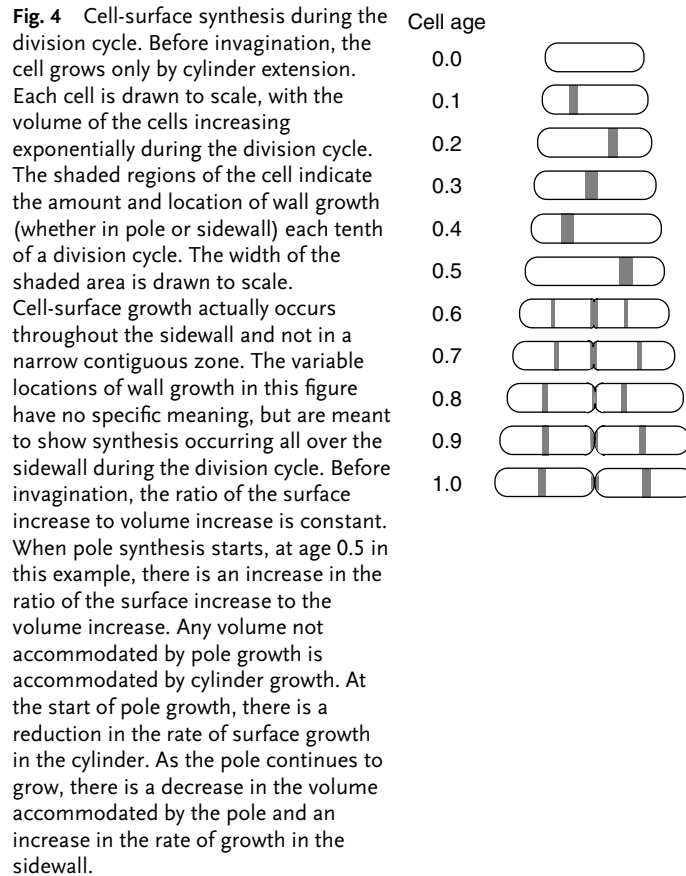#### 3.3.1 Peptidoglycan Synthesis during the Division Cycle

The cell surface is made to perfectly enclose, without excess or deficit, the cytoplasm synthesized by the cell. Cell cytoplasm increases continuously during the division cycle (described above), and therefore the cell surface is made continuously. How is the bacterial cell surface made and duplicated during the division cycle? What is the rate and topography of cell-surface synthesis during the division cycle?

Consider an imaginary cell in which the cytoplasm is enclosed in a cell-surface tube that is open at each end; the cytoplasm remains within the bounds of the tube. The cytoplasm in the newborn cell is thus encased in the cylinder of cell surface that is made up of membrane and peptidoglycan. As the cytoplasm increases exponentially (see above), the tube length increases to exactly enclose the newly synthesized cytoplasm. In this imaginary case, the cell surface increases exponentially in the same manner as the cytoplasm. When the cell cytoplasm doubles, the tube divides into two new cells and the cycle repeats. In this imaginary cell, the cytoplasm increases exponentially, the internal volume of the cell increases exponentially, the surface area increases exponentially, and the density of the cell, that is, the total weight of the cell per cell volume, is constant during the division cycle.

However, a real rod-shaped cell does have ends, and therefore the pattern of cell-surface synthesis during the division cycle is not simply exponential. If the cell surface were synthesized exponentially, the cell volume cannot increase exponentially. Since the cytoplasm increases exponentially, there would have to be a change in cell density. (In theory, it may be that major changes in cell shape could allow a pure exponential increase in cell mass and cell surface, but such changes are clearly not observed during the cell-division cycle.) Cell density, however, is constant during the division cycle. A proposal for cell-surface synthesis that allows an exponential increase in cell volume, and therefore a constant cell density, is presented in Fig. 4. Before invagination, the cell grows only in the cylindrical sidewall. After invagination, the cell grows in the pole area and the sidewall. Any volume required by the increase in cell cytoplasm that is not accommodated by pole growth is accommodated by an increase in sidewall. The cell is considered a pressure vessel, and when the pressure in the cell increases, there is a corresponding increase in cell surface area. The pole is preferentially synthesized and any residual pressure due to new cell cytoplasm that is not accommodated by pole growth is relieved by an increase in cylindrical sidewall area.

The resulting pattern of synthesis is approximately exponential. The formula describing surface synthesis during the division cycle is a complex one, including terms for the shape of the newborn cell, the cell age at which invagination starts, the pattern of pole synthesis, and the age of the cell. One consequence of this model of surface synthesis is that at no time is surface synthesis exponential, since the rate of surface synthesis is not proportional to the amount of surface present over any period of time.

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Fig. 4 Cell-surface synthesis during the division cycle. Before invagination, the cell grows only by cylinder extension. Each cell is drawn to scale, with the volume of the cells increasing exponentially during the division cycle. The shaded regions of the cell indicate the amount and location of wall growth (whether in pole or sidewall) each tenth of a division cycle. The width of the shaded area is drawn to scale. Cell-surface growth actually occurs throughout the sidewall and not in a narrow contiguous zone. The variable locations of wall growth in this figure have no specific meaning, but are meant to show synthesis occurring all over the sidewall during the division cycle. Before invagination, the ratio of the surface increase to volume increase is constant. When pole synthesis starts, at age 0.5 in this example, there is an increase in the ratio of the surface increase to the volume increase. Any volume not accommodated by pole growth is accommodated by cylinder growth. At the start of pole growth, there is a reduction in the rate of surface growth in the cylinder. As the pole continues to grow, there is a decrease in the volume accommodated by the pole and an increase in the rate of growth in the sidewall.

Cell age
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

As with cytoplasm and DNA synthesis, we can derive the cell-cycle pattern of surface synthesis from our understanding of the molecular aspects of peptidoglycan synthesis. The peptidoglycan sacculus of the cell is composed of glycan strands encompassing the cell, perpendicular to the long axis, as shown in Fig. 5. These strands are cross-linked by peptide chains. Because the cross-linking in the load-bearing layer is not complete, one can have new strands in place, below the taut load-bearing layer, prior to the cutting of the bonds linking old strands. As shown in Fig. 5, the load-bearing layer is stretched by the turgor pressure in the cell. An infinitesimal increase in cytoplasm leads to an infinitesimal increase in the turgor pressure of the cell. This increase in turgor pressure places a stress on all of the peptidoglycan bonds, thus reducing the energy of activation for bond hydrolysis. The result is that there is an increase in the cutting of stressed bonds between the glycan chains. When a series of cuts is made, allowing the insertion of a single new strand into the load-bearing layer, there is an infinitesimal increase in cell volume. This increase in volume leads to a reduction of the stress on the remaining bonds. By a continuous repetitive series of cytoplasm increases, surface stresses,
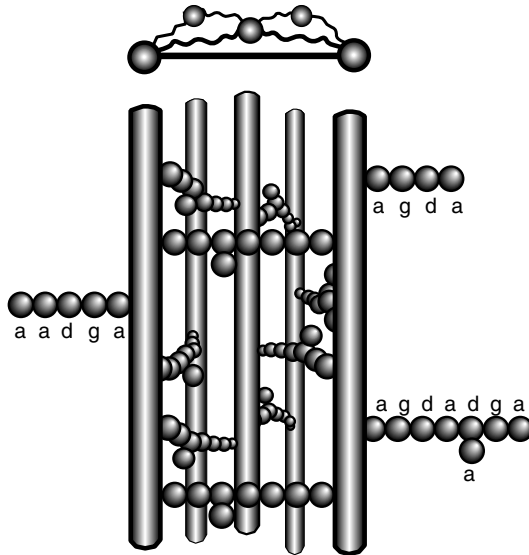
Fig. 5 Three-dimensional representation of peptidoglycan structure. This is an idealized representation of peptidoglycan structure as seen from the outer surface. The thick bars represent glycan chains at the outside of the peptidoglycan layer. The thinner bars represent glycan chains below the outer layer. The stretched chains of circles represent amino acids cross-linking the glycan chains. The chains below the stretched surface of the cell rise to the outer layer when the taut layers of the peptidoglycan are hydrolyzed. The figure shows a cross-sectional view through the glycan chains illustrating the taut outer layer and the more loosely inserted inner material. The letters indicate the amino acids composing the cross-links; a = alanine, d = diaminopimelic acid, and g = glutamic acid, with italicized letters present in the D-configuration.

enzymatic cuts, and volume increases, one gets an increased cell volume that precisely accommodates the increase in cell cytoplasm.

An inside-to-outside mode of peptidoglycan growth, similar to that for *Bacillus subtilis*, has been proposed for *E. coli*. This proposal is based on the observed recycling of murein, the calculated amount of peptidoglycan per cell, and the existence of trimeric and tetrameric fragments that are consistent with a multilayered peptidoglycan structure. The insertion of new peptidoglycan strands in an unstressed configuration prior to their movement into the load-bearing layer of the peptidoglycan can explain all of these observations. The recycling of peptidoglycan may be a strain-specific result, as there is no major release or recycling of peptidoglycan in *Salmonella typhimurium* or in *E. coli* B/r. At this time, the inside-to-outside mode of surface growth cannot be excluded.

Although this discussion of the rate of surface synthesis during the division cycle has dealt primarily with peptidoglycan, it applies equally to membranes and other surface-associated elements. The cell membrane grows in response to the increase in peptidoglycan surface and coats the peptidoglycan without stretching or buckling. The area of the membrane increases in the same way in which bacterial peptidoglycan increases.

One must make distinctions among cell components with regard to their location in the cell, rather than with regard to their chemical properties. Cell proteins can be divided into two categories – those associated with the cytoplasm and those associated with the surface. Proteins associated with the membranes are included in the surface category of synthesis during the division cycle. If a bacterial cell had a histone-like protein associated with DNA, there would be a third category of protein-synthetic patterns, proteins synthesized

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during the division cycle with constant rates of synthesis. Proteins are synthesized during the division cycle with a pattern that is presumably consistent with their final location or category. Proteins are not a monolithic group nor are they divided into a large number of groups. The three-category system proposed here allows us to conceptualize the possible patterns of protein synthesis observed during the division cycle.

3.3.2 The Invagination Process

The division of the bacterial cell into two daughter cells occurs by the formation of two new poles in the middle of the dividing cell. As there are very few DNA-less cells produced during balanced growth, the division process must be coordinated with the replication process so that division does not occur until two genomes are present. The invagination process that forms two poles in the middle of the cell involves a complex process whereby there is an inward growth of the peptidoglycan and the associated membrane of the cell surface.

One of the earliest events related to the invagination process is the localization of the FtsZ protein to the future site of pole formation. The FtsZ protein is localized to the division site in a pattern called the *FtsZ ring*. The FtsZ protein accumulates at the mid-cell site where division will occur before any visible invagination. During the invagination process, the FtsZ protein is located at the leading edge of the invagination process. Thus, the FtsZ ring continues to define a ring of decreasing size as invagination proceeds.

Other proteins necessary for invagination and division are apparently localized at the center of the cell because of their association with FtsZ.

3.3.3 FtsZ Protein Is Involved in Septum Formation and Cell Division

Recent results indicate that the tubulin-like FtsZ protein plays a central role in cytokinesis or cell division as a major component of a contractile cytoskeleton. An indication that the FtsZ protein is the key initiator of septation is that mutants of this protein that form filaments show no indication of any constriction. Other septation mutants presumed to be later in the pathway of septum formation do show some indication of septation. The FtsZ protein resembles the eukaryotic tubulin molecule and this similarity has led to speculations that the FtsZ protein has cytoskeletal properties that allow it to form the new pole in the middle of the cell.

3.3.4 Localization of the Division Site

The actual mechanism by which the division site is localized at mid-cell position is unknown. One model of the division-site localization proposes that the site exists prior to any observation of FtsZ localization. An alternative model proposes that the division site is determined in some way by the reaction of the cell surface to the presence of DNA; the absence of DNA in the center of the cell would thus lead to invagination-site localization.

An understanding of site location is strongly influenced by the existence of minicells. If invagination occurs, upon occasion, near a pole rather than in the center of a cell, a very small DNA-less "minicell" is produced that is essentially two poles without an intervening sidewall. The genetics of minicell formation is complicated, but the main idea is that the products of the min genes have a negative function and normally inhibit abnormal invagination sites. Thus, inactivation of any of a number of min genes leads to minicell

formation owing to the inappropriate loca-tion of invagination sites.

4
Plasmid Replication during the Division Cycle

4.1
High-copy Plasmids

If a cell has a large number (20–100) of plasmids per newborn cell, then one can consider that these plasmids replicate during the division cycle in proportion to those present (i.e. exponentially) and then assort themselves randomly into the newborn cells. Because the plasmid number is high, there is little chance of one of the daughter cells ending up with no plasmids and with all of the plasmids segregating to the other daughter cell. This pattern appears to be the case for naturally occurring, high-copy number plasmids.

One possible exception is the artificial plasmid made by cloning the origin (oricC) region of the bacterial chromosome. This appears to have a random assortment mechanism, but it is synthesized at a precise time during the division cycle that coincides with the normal time at which the chromosome initiates replication.

4.2
Low-copy plasmids

Low-copy plasmids are usually larger than high-copy plasmids and are present on the order of 1 to 2 per cell. Examples of such plasmids are the F-factor and the P1 plasmid. These replicate in a precise way at a particular time during the division cycle. The rules of their replication are similar to that regulating chromosome replication, with initiation of plasmid replication occurring when a fixed amount of cell mass is present per plasmid origin.

4.3
Minichromosome Replication during the Division Cycle

Minichromosomes replicate at the same time as normal chromosome initiation. The behavior of minichromosomes is paradoxical in that in a cell with numerous additional origins of replication there would be a competition for the "initiator" of replication. Such a competition would lead to abnormal cell sizes or some alteration in cell growth pattern. No such alteration is observed.

5
Segregation of Cell Components at Division

Producing the right amount of material so that the cell can divide is not enough for a successful division cycle. At division, the material in the mother cell must also be properly apportioned to the newborn cells. In a cell that divides to produce two equivalent daughter cells, the segregation problem is to ensure that both cells have the same amount of each portion of the cell material. Let us see how the three categories of material in the mother cell are segregated properly to the new daughter cells.

5.1
Segregation of Cytoplasm

The cytoplasmic components of the cell appear to segregate randomly at division. There does not appear to be any compart-mentalization or hindrance to segregation of cytoplasm (Fig. 6).

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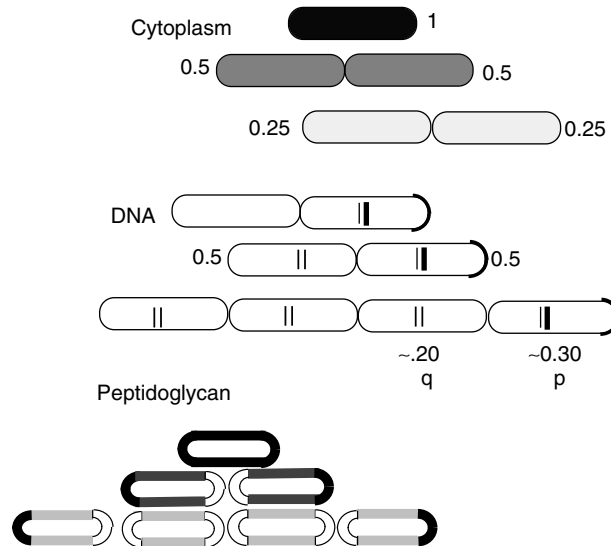
Fig. 6 Segregation of cytoplasm, surface, and DNA at division. The cytoplasm of the cell is segregated randomly at division. Each daughter cell shares equally in the parental cytoplasm (ribosomes, soluble proteins, ions, etc.), which can be considered randomly distributed throughout the dividing cell. The DNA is segregated nonrandomly, but each daughter cell gets a complete and equal complement of DNA from the dividing cell. The nonrandom segregation is at the level of a single strand. Since each DNA duplex is made up of an ''older'' strand and a new complement made on the older strand, and each cell is composed of an ''older'' pole and a pole just made at the previous division, one can see whether strands of one age go preferentially toward one or the other age pole. Experimental analysis indicates that the DNA strands segregate as though the older DNA strand goes preferentially toward the pole that it went to at the previous division. This may be phrased as the newer strand goes toward the newer pole. The degree of nonrandom segregation is not perfect, but is stochastic, and is approximately 60/40 rather than 50/50. The surface of the cell segregates to the daughter cells at division such that the poles are conserved and the sidewall segregates with no apparent conservation of sidewall peptidoglycan. It is not clear whether insertion of new wall material over the side is perfectly uniform or whether there is some nonuniform pattern of insertion.

5.2
Segregation of DNA

DNA segregates so that each daughter cell receives a complete genome from the dividing mother cell (Fig. 6). As the DNA nucleoid is relatively compact within a dividing cell, if the nucleoids were apportioned completely randomly, one might expect that at some divisions one of the daughter cells would not receive any DNA and the other daughter cell would

receive two genomes. That this is a rare event supports the notion that segregation of the DNA is a regulated and deterministic process that leads to very few nonnucleated cells in a culture.

In addition to the segregation of individual nucleoids, the separate strands of the DNA have rules that describe their segregation and which are compatible with the deterministic equipartition of DNA to the two daughter cells. As all rod-shaped cells are asymmetric in that one of the poles is the new pole (produced at the last division) and one pole is necessarily older, one can see how older DNA strands and newer DNA strands (the newer strands having just been made on an older, pre-existing strand) segregate with regard to pole age. The general rule is that the older strands segregate slightly preferentially to the older poles and the younger strands segregate preferentially to the newer or younger poles. This phenomenon is consistent with other data that indicate some attachment of the DNA to the cell surface.

5.3
Segregation of the Cell Wall

The peptidoglycan is a large macromolecule that grows by intercalation of new material between old material. Since all the peptidoglycan is covalently linked in one macromolecule, there is no ability for the peptidoglycan components to move with respect to each other. The segregation of cell wall material is therefore determined by the location of the cell wall material. Once in place, the wall segregates into the newborn cells so that the old poles go to each cell, and the sidewall is segregated to the newborn cells (Fig. 6).

6
Are There Events during the Division Cycle?

There are only four discrete events that occur during the division cycle of a growing rod-shaped bacterial cell. They are (1) the initiation of DNA replication, (2) the termination of DNA replication, (3) the initiation of pole formation, and (4) the completion of pole formation. Other aspects of cell growth are too continuous to be considered cell-cycle-specific events. For example, the addition of new nucleotides at position 43,567 on the chain of DNA may be considered a unique event, and one that occurs at a particular time in the division cycle, but because of cell-cycle variability, the time of this synthetic occurrence can never be precisely obtained. The time of insertion of a particular nucleotide pair has no meaning for the cell cycle; only the initiation and termination of DNA replication are important. With regard to the division cycle, the DNA is an amorphous material with no biochemical specificity along the strand.

Similar considerations apply to the cell surface and the cytoplasm. No aspect of peptidoglycan strand insertion is unique in time during the division cycle. A new strand is inserted at random sites in response to the stretching of the cell surface. This may occur in one cell at position 0.376 of the cell length; at the same time, in another cell, the new strand may be inserted at position 0.549. For an analysis of the cell cycle, we should consider pole and sidewall growth as uneventful extensions of the cell surface. Regarding the cytoplasm, one may consider the increase in ribosome content from 37,411 to 37,412 an event; but such an individual event is not measurable and is without meaning in the cell cycle. Thus, cytoplasm synthesis,

DNA synthesis, and cell-surface synthesis are uneventful during the division cycle.

In contrast to our understanding of the principles of cytoplasm, cell-surface, and DNA synthesis, the biochemical mechanisms of the four events occurring during the division cycle – the initiation and termination of DNA synthesis and the initiation and cessation of pole formation – are still unknown. While a great deal is known about the biochemistry of initiation of DNA replication, the mechanism by which this initiation is regulated is less well understood. In addition, very little is known of the biochemical principles involved in either the termination of DNA replication or the start and cessation of pole formation. A cell-surface structure, the periseptal annulus, has been proposed as a possible first step in the formation of a new pole. If the periseptal annulus is the start of pole formation, the important question is whether there are definable steps between the formation of the periseptal annulus and the start of invagination.

7

Do Checkpoints Control the Bacterial Cell Cycle?

In eukaryotic cell-cycle studies, the concept of "checkpoints" looms large. Checkpoints are the postulated functions of a cell that determine that one function is properly completed before a subsequent function occurs. Thus, in eukaryotic cells it is postulated that a checkpoint function determines that DNA has replicated completely and properly before allowing mitosis to occur. The checkpoint is thus a valuable function that prevents abnormal functions from occurring.

Studies of this type of phenomenon in bacteria can provide a corrective lens for understanding or more rigorously defining checkpoints. In bacteria, if DNA synthesis is inhibited, cells with only one genome (i.e. cells that have not completed replication of a total chromosome) do not divide. Even when DNA is inhibited or damaged, a cell with two genomes will divide, while a cell with one genome will not. Thus, in bacteria, DNA damage *per se* does not activate a checkpoint to prevent division. It is believed that the presence of a single genome in the center of a cell prevents division by its central presence, not the activation of a function that checks for completion of DNA replication.

The bacterial experience thus suggests that a distinction should be made between the existence of a function external to the tested function (i.e. some activity that checks on whether DNA replication has been completed) and the actual function itself acting as a brake on further activity. The failure to complete DNA replication is the inhibitor of division, and there is no checking by some "checkpoint" activity.

A similar analysis may be applied to the ubiquitous postulation of restriction points in eukaryotic cells. Restriction points are points or events postulated to exist in the G1-phase of the division cycle. Passage of a cell through the restriction point is a requirement for entering the S-phase and eventual division. The restriction point was defined by the effect of different starvation regimens on DNA replication and the resumption of DNA replication following release from starvation.

It is interesting that a bacterial restriction point was postulated to exist before the proposal of the eukaryotic restriction point, although the term *restriction point* was not used. Subsequent analysis of the bacterial restriction point indicated that the proposed control point was an experimental artifact related to leakage of

synthesis under starvation conditions. The eukaryotic restriction point is subject to the same critique.

8
Temporal Variation of the Bacterial Division Cycle

Division cycles in a culture are not precisely timed. There is a great deal of variability in division cycles, with a culture having a 60-min doubling time probably having cells with interdivision times between 40 and 100 min. The variability of the division cycle can be visualized as due to the variability of the separate components that make up the division cycle. Thus, for two identical newborn cells one can have the subsequent division occurring at different times because of (1) variability in mass synthesis in the two cells leading to variation in the time of initiation of DNA replication, (2) variation in the rate of DNA replication in the two cells, (3) variation in the time between termination and division, (4) and variation in the equality of division (which leads to variation in the next division cycle).

9
Alternative Bacterial Division Cycles

9.1
Caulobacter crescentus Division Cycle

At first glance, the division cycle of the stalked bacterium *Caulobacter crescentus* appears quite different from that of *E. coli*. At division, two different-sized and different looking cells are produced (division is not in the middle of the mother cell and one cell is stalked and the other is flagellated), the DNA patterns during the division cycle differ in each

of the daughter cells, and there are cell-cycle-specific syntheses of flagellum-related materials. The most important difference is that the poles of the daughter cells are different in that one cell has a flagellum and the other has a stalk. Because of the production of two different appearing cells, the growth and division of *Caulobacter* has been described as a model of cell differentiation.

The division cycle of *Caulobacter* can be shown to correspond to that of the standard *E. coli* model by considering that the different DNA patterns are due to the different sizes of the two daughter cells arising by division not occurring in the middle of the dividing mother cell, and that the cell-cycle-specific pattern of expression of flagellum-related genes is related to the completion of poles after the act of cell division. Flagellum synthesis is thus considered a part of surface synthesis. The cycle-specific pattern observed in cells is related more to the window of time in which poles are made rather than synthesis at a particular time during the division cycle (Fig. 7).

9.2
Bacillus subtilis Division Cycle

The division cycle of the gram-positive bacterium *Bacillus subtilis* is relatively similar to that of *E. coli*. The major difference between the organisms lies in the fact that the *B. subtilis* cell wall is much thicker (giving rise to its gram-positive character), and this requires an inside-to-outside pattern of peptidoglycan growth. The cell wall is made adjacent to the cytoplasmic membrane, and this wall layer moves out as subsequent layers are made between it and the cytoplasmic membrane. This leads to the multilayered *B. subtilis* peptidoglycan.

Fig. 7 The *Caulobacter* cell cycle. *Caulobacter* cells divide unequally, producing a smaller swarmer cell that is motile and a larger stalked cell that is generally attached to a surface by the stalk. As drawn here, the development of *Caulobacter* can be followed by concentrating on the two new poles formed by the first division. The development of the poles in both cells is a succession from bald, to flagellated, to stalked. Because the swarmer cell is smaller the development is slightly delayed, suggesting a size control for both the initiation of DNA replication and the completion of pole development. As drawn here, the cell-cycle-specific synthesis of flagella protein (flagellin) is a result of pole development following cell separation. The ultimate end of pole development is a stalk, after which no further changes in the pole occur. Thus, with regard to the general model of cytoplasm synthesis proposed here, the *Caulobacter* cytoplasm (excluding surface) does not exhibit cell-cycle-specific patterns of gene expression.

9.3
Streptococcal Growth during the Division Cycle

The growth of spherical bacteria such as Streptococci reveals a different pattern of surface growth. There is no apparent intercalation of new peptidoglycan material into the preexisting older peptidoglycan. Rather, the old peptidoglycan is conserved and new peptidoglycan grows at the center of the cell to make a completely new cell pole.

10
Mass Increase Is the Driving Force of the Division Cycle

The driving force of the division cycle is cytoplasm synthesis. Some part of the energy used by the cell to make cytoplasm drives the biosynthesis of the cell surface by causing stresses along the cell surface and this stress leads to the breaking of peptidoglycan bonds. The insertion of new peptidoglycan then leads to the increase in cell size. Cytoplasm increase also regulates DNA synthesis. Cell size at birth is greater at faster growth rates (Fig. 3) because the initiation of new rounds of DNA replication occurs when the cell has a unit (or critical) amount of cytoplasm per origin of DNA. Cell size at initiation is proportional to the number of replication points in the cell, and that is why cells are larger at faster growth rates; at faster growth rates there are more origins per cell at initiation. The cell "titrates" the amount of cytoplasm or some specific molecule that is a constant fraction of the cytoplasm. Thus, DNA synthesis is initiated at all available origins when the amount of cytoplasm per origin reaches a particular value.

The cell cytoplasm increases as fast as it can, given the external nutrient conditions. The synthesis of DNA and cell

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surface cannot outpace or lag behind cytoplasm synthesis. DNA synthesis cannot go faster because it is waiting to initiate new rounds of DNA replication in response to cytoplasm synthesis nor can it go slower because the cytoplasm would increase without initiating new rounds of DNA replication. Cell surface is made to just enclose the newly synthesized cytoplasm. The regulation of surface and DNA synthesis by cytoplasm thus explains why there is no dissociation of these syntheses during the division cycle and during the growth of a culture. Furthermore, the failure to complete DNA replication (so that a cell has only one genome) prevents cell division; the cell thus ensures that there will be enough material (genome, cytoplasm, and cell surface) to allow the production and survival of two new daughter cells.

11

The Bacterial Growth Law during the Division Cycle

It has been speculated that there is a "bacterial growth law" that can be discovered by sensitive methods of analysis. Does the cell grow linearly, bilinearly, exponentially, or perhaps follow some other, yet undiscovered, growth law? If there were a general law of cell growth, independent of the biosynthetic patterns of the three categories that comprise the cell, then the individual categories of biosynthesis would have to accommodate themselves to this overall growth law.

There is no simple mathematical growth law regulating or describing bacterial growth during the division cycle. Bacterial growth during the division cycle is the simple weighted sum of the biosynthetic processes that are described by the three categories proposed here. As presented in Table 1, it can be seen that the components of the cytoplasm of the cell are 80% of the total cell weight. This means that the growth of the cell is approximately exponential. A slight deviation from exponential growth is due to the contribution of cell surface and DNA synthesis. Cell growth, then, is the result of a large number of individual reactions, regulated by local conditions, and not conforming to any overriding growth law.

Tab. 1 Composition of *Escherichia coli*, a typical gram-negative, rod-shaped bacterium.

Cell component	Molecules per cell	Percent dry weight
Cytoplasm		
Protein	2,350,000	55.0
RNA	255,480	20.5
Polyamines	6,700,000	1.1
Glycogen	4,300	3.5
Metabolites, ions, cofactors		2.5
Surface		
Peptidoglycan	1	2.5
Lipid	22,000,000	9.1
Lipopolysaccharide	1,430,000	2.4
Genome		
DNA	2.1	3.1

If one wished to state the growth law, the growth law is simply the sum of the individual synthetic patterns of the three categories of the cell material.

12
Experimental Analysis of the Bacterial Division Cycle

How can one study the pattern of synthesis of cell components during the division cycle? At first thought, it appears that one could study the division cycle by preparing a synchronized bacterial culture – that is, a culture composed of cells all of the same age, for example, newborn cells – and taking measurements of the cell components at different times during the division cycle. It turns out that this method has rarely, if ever, led to any concrete results. This is presumably due to the introduction of perturbations and artifacts when cells are synchronized, as no naturally synchronized populations are available.

The methods that have given results are nonperturbing, nonsynchrony methods. One is the membrane-elution method, or "baby machine," which analyzes the division cycle of unperturbed cells. In this method, a label is added to a growing culture, the labeled cells are filtered onto a membrane, the cells bind as though bound at one end or pole, and when medium is pumped through the inverted membrane, newborn cells are eluted from the bound cells. Since the newborn cells arise from the bound cells as a function of the original cell age, the first newborn cells arise from the oldest cells at the time of labeling. With time, the newborn cells arise from cells labeled at younger and younger cell ages. By measuring the amount of label per cell during elution, one can determine the pattern of synthesis during the division cycle. This has been done successfully for DNA, plasmids, protein, RNA, peptidoglycan, and cell membrane.

Another method for cell-cycle analysis is flow cytometry, where the amount of material per cell in a cell population is determined for individual cells by a complex system of liquid handling and laser-excitation engineering. The quantitative distribution of cell material can give the relative rate of synthesis during the division cycle. This method has been successful for DNA replication during the division cycle and has confirmed the initial results obtained with the membrane-elution method. It is not clear whether the method is sensitive enough for bacteria to enable the discovery of a particular pattern of DNA replication.

13
Genetic Analysis of the Bacterial Cell Cycle

Complementing the physiological and biochemical analyses of the bacterial division cycle has been a large amount of work on isolating and analysing mutants that affect the division cycle. Because a successful division requires that the cell make all of its required material, a mutation affecting the synthesis of any required material is, in some trivial sense, a mutation affecting the division cycle. Thus, a mutant unable to make a particular amino acid, such as leucine, that is required for growth would stop progressing through the division cycle. However, this would be a trivial relationship to the division cycle because the leucine is required throughout the division cycle and is not related to a specific control mechanism.

More interesting are those mutants that affect the major events of the division cycle

such as the start of DNA synthesis and the start of septation. A large number of mutations affecting these processes have been isolated, and their study has revealed that a number of enzymes and proteins are required for cell-surface synthesis and DNA synthesis.

14
Die Einheit der Zellbiologie

Are the interrelationships of cell-cycle regulation, proposed here for *E. coli*, applicable to other organisms? Other regulatory mechanisms have been proposed in other cell types. For example, a circular regulatory system was proposed for *Caulobacter* and a stochastic mechanism as well as a rate-controlled model for animal cells. Is the mechanism of cell-cycle regulation subject to historical accident? Or is there a unity of mechanisms regulating the division cycle of all types of cells? I conjecture that the *E. coli* model of cell-cycle regulation is generally applicable to other organisms, and that the optimal design of the cell cycle is exhibited by the *E. coli* cell cycle.

In the second decade of this century, Kluyver and his colleagues explicitly proposed the principle of the Unity of Biochemistry, or as originally proposed, Die Einheit der Biochemie (the oneness of biochemistry). Although the original proposal dealt primarily with the nature of oxidation–reduction reactions, the basic principle has evolved to encompass even more unifying principles. The nature of the gene, the genetic code, the mechanism of protein synthesis, the use of enzymes for biochemical changes, the patterns of enzyme regulation, and all of the fundamental aspects of biochemistry appear similar throughout the different organisms on earth. While there are exceptions and unique aspects to many organisms, the essential principle that there is a unified biochemistry has been supported.

Here we must now add, with regard to the cell cycle, the Unity of Cell Biology – *Die Einheit der Zellbiologie* – that is analogous to the Unity of Biochemistry. Phenomena suggesting the existence of other modes of cell-cycle regulation in animal cells have been shown to be consistent with the model for the bacterial division cycle proposed here. For example, the constancy of C- and D-periods is mimicked by the constancy of their analogues in animal cells, the S and G2 periods. The variation in cell size with growth rate and the rules regarding size determination also appear to hold for all organisms. The rules of the cell-division cycle – that is, the logic and design principles of the division cycle – are the same for *Escherichia* and escargot, *Salmonella* and salmon. Although there are many detailed differences between eukaryotic and prokaryotic division cycles, at the deepest level the principle of the Unity of Cell Biology proposes that the division cycle is ultimately regulated by the continuous accumulation of some molecule that is titrated against a fixed amount of another cell component. From this viewpoint, cell-cycle-specific events identified in eukaryotic cells are the result or symptom of a deeper regulatory principle that is produced in a cell-cycle-independent manner. The question for future analysis is whether there is some deeper rule that ensures this common pattern in all cells. I conjecture that this is indeed the case and hope that a search for the underlying meaning of size control in all cells is now the new goal of cell-cycle research.

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