THE *ESCHERICHIA COLI* CELL CYCLE

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Between the birth of a cell and its subsequent division, all of the cell components double. At what rate is material synthesized during the division cycle and how does the cell ensure that there is a precise doubling of cell components? How are these syntheses related to an orderly progress of synthesis through the division cycle? Is there a bacterial growth law? I will answer these questions by describing cell growth in terms of the biosynthesis of three categories of material: cytoplasm, genome and cell surface.

The aggregation problem.

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. For example, the economy of an individual country is often aggregated into a single number, the gross national product. We must now consider the aggregation problem for the analysis of the bacterial division cycle.

In the analysis of the bacterial division cycle, the question arises as to how one aggregates the different parts of the cell 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 a different category from that of peptidoglycan? Are the enzymes involved in macromolecule metabolism synthesized differently from those involved in the small molecule metabolism? 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.

With regard to synthesis during the bacterial division cycle, I propose that there are 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 (Cooper, 1988c). The first category is the cytoplasm, which is the entire accumulation of enzymes, 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. The second category is the genome, the one-dimensional linear DNA structure. For our understanding of biosynthesis, it is the linear aspect of DNA that is important, although the folded genome is a three-dimensional object. 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 cytoplasm, genome, and cell surface.

Cytoplasm synthesis during the division cycle.

Consider a unit volume of cytoplasm. It contains all of the enzymes required for the breakdown of nutrients, the biosynthesis of small molecular precursors of macromolecules and 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 exponential pattern is illustrated in figure 1.

Experimental measurements confirm the exponential biosynthesis of cytoplasm during the division cycle (Cooper, 1988b; Dennis, 1971a,b; Lutkenhaus *et al.*, 1979). These experiments indicate that the synthesis of cytoplasm is independent of the division cycle. There are no changes in the specific rate of synthesis or pattern of cytoplasm synthesis that are related to any particular cell-cycle event. 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 part 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.

Despite arguments that have been made for other modes of cytoplasm synthesis, it is proposed here that cytoplasm synthesis occurs without reference to cell age. Papers have appeared periodically claiming to show that one or another protein is made at some particular point during the division cycle, or that there is an abrupt change in the synthetic rate during the division cycle (see Cooper (1988b) for references). Experiments suggesting variations in cytoplasm synthesis during the division cycle have used synchronized cultures, and synchronized cultures have the ability to produce artifacts. The

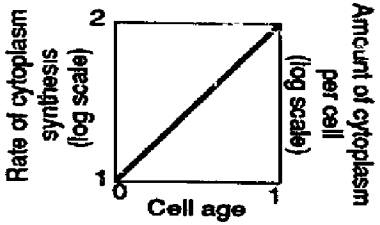

FIG 1 — *Cytoplasm synthesis during the division cycle*

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

evidence for a smooth, continuous and exponential increase in cytoplasm comes from experiments that use the membrane-elution method (Cooper, 1989b). This method, sometimes called the "baby-machine", has led to the acceptance of a particular pattern of macromolecule synthesis during the division cycle (Cooper and Helmstetter, 1968; Helmstetter and Cooper, 1968). The pattern of DNA synthesis during the division cycle, recently shown to be correct by flow-cytometric analysis (Skarstad *et al.*, 1985), was 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 argument, 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 making 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 was *not* activated for synthesis during the division cycle, but instead was recruited for biosynthesis only at the instant of division, one would have linear cytoplasm synthesis during the division cycle. Although this possibility exists, I know of no mechanisms which allow 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. Until such a mechanism is found, it will be difficult to accept non-exponential modes of cytoplasm synthesis.

DNA synthesis during the division cycle.

The DNA synthesis pattern is comprised of one or more periods of constant rates of DNA accumulation (Cooper and Helmstetter, 1968; Helmstetter and Cooper, 1968). In cells with a 60-min interdivision time (fig. 2), there is a constant rate of DNA synthesis for the first 40 min, 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 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.

The constant rates of DNA synthesis can be understood in terms of the molecular aspects of its synthesis. DNA is synthesized by the movement of a growing point along the parental double helix leaving two double helices in its wake. The rate of movement of a growing point can be considered invariant and independent of its location in the genome. This means that the rate of DNA synthesis is proportional to the number of growing points and the number of growing 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 pattern of constant rates of synthesis is derived from, and con-

sistent with, our understanding of the biochemistry of DNA synthesis.

Surface 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, and therefore 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 which is open at each end; the cytoplasm remains within the bounds of the tube. The cytoplasm in the newborn cell is encased in the cylinder of cell surface which is made up of membrane and peptidoglycan. As the cytoplasm increases exponentially, the tube length increases to exactly

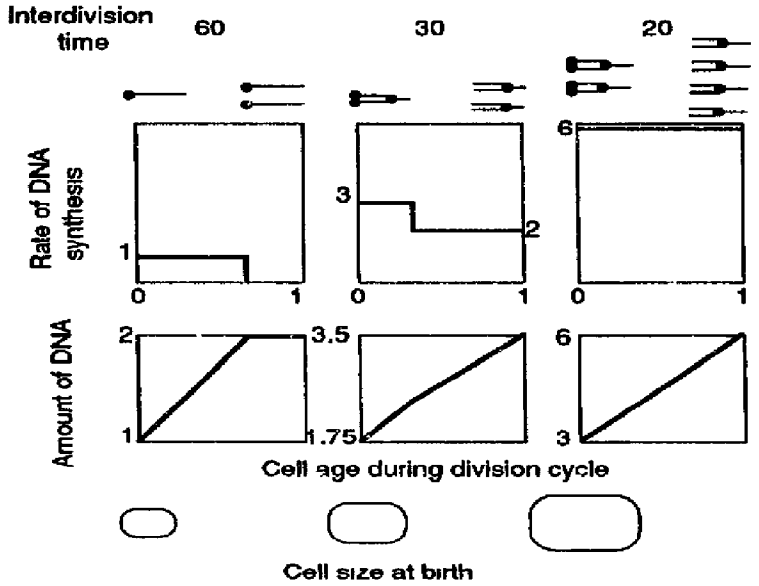

FIG 2. — *DNA synthesis during the division cycle*

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 are 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 are also in the ratio of 1/2/4.

enclose the newly synthesized cytoplasm. 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, *i.e.*, the total weight of the cell per cell volume, is constant during the division cycle. Further, the ratio of the rate of cytoplasm to the rate of cell surface synthesis is constant throughout the division cycle. But 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 could not increase exponentially. Since the cytoplasm increases exponentially, there would have to be a change in cell density. Cell density, however, is constant during the division cycle (Kubitschek, 1987). A proposal for cell-surface synthesis that allows an exponential increase in cell volume, and therefore a constant cell density, is presented in figure 3. Before invagination, the cell grows only in the cylindrical side-wall. After invagination, the cell grows in the pole area and the side wall. Any volume required by the increase in cell cytoplasm that is not accommodated by pole growth is accommodated by an increase in side-wall. The cell is considered a pressure vessel (Koch, 1983), 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 side-wall area (Cooper, 1988a; Cooper and Hsieh, 1988).

The resulting pattern of synthesis is approximately exponential (fig. 3). 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 (Cooper, 1988a). It is less difficult, however, to comprehend the pattern of surface synthesis by measuring the ratio of the rate of surface synthesis to the rate of cytoplasm synthesis. Before invagination, cell surface growth occurs only by cylindrical extension. As the radius of the cell is constant during the division cycle (Cooper, 1989a), the rate of surface synthesis prior to invagination is directly proportional to the rate of cytoplasm synthesis. After invagination, there is an increase in the rate of surface synthesis relative to the rate of cytoplasm synthesis (Cooper, 1988a). The relative increase of surface to volume is greater during pole formation than during side-wall formation. This view of cell surface synthesis has led to the powerful "ratio-of-rates method" where one measures the ratio of the rate of surface synthesis to the rate of cytoplasm synthesis, rather than the rate of surface synthesis alone. This ratio is constant during the first part of the division cycle, when there is only side-wall growth, and increases during invagination (fig. 3). This pattern of cell surface synthesis has been observed in *Salmonella typhimurium* (Cooper, 1988a) and *Escherichia coli* (Cooper and Hsieh, 1988). 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 for any period of time.

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 comprised of glycan strands encompassing the cell perpendicular to the long axis. These strands are cross-linked by peptide chains. Because the cross-linking is not complete, one can have new strands i. place prior to the cutting of the bonds linking old strands. 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 which reduces the energy of activation for bond

hydrolysis. The result is that there is an increase in the cutting of stressed bonds. When a series of cuts is made, allowing the insertion of a single new strand into the load-bearing layer (Cooper, Hsieh and Guenther, 1988), there is an infinitesimal increase in cell volume. This increase in volume leads to a reduc-

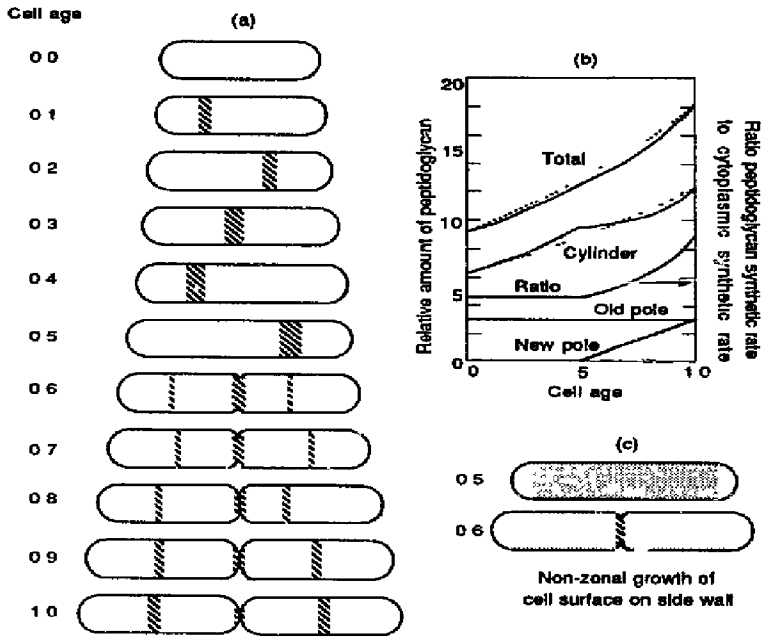

FIG. 3. — *Cell surface synthesis during the division cycle.*

The cells at the left (a) are drawn with a cylinder length of 2.0 and a radius of 0.5. 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 side wall) each tenth of a division cycle. The width of the shaded area is drawn to scale Cell surface growth actually occurs throughout the side wall and not in a narrow contiguous zone. Before invagination, the ratio of the rate-of-surface-increase to the rate-of-volume-increase is constant When pole synthesis starts, at age 0.5, there is an increase in the ratio of the rate-of-surface-increase to the rate-of-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 side wall. At the upper right (b) is a plot of the expected pattern of accumulation of peptidoglycan or cell surface during the division cycle. The total accumulation of peptidoglycan is the sum of the individual accumulations of new pole, old pole and the cylindrical side wall. The dotted line represents the expected pattern for exponential synthesis The ratio of the rate-of-surface to the rate-of-cytoplasm synthesis is indicated. At the lower right (c), an explicit illustration of the dispersive, non-zonal growth of the side wall is illustrated for cells of ages 0.5 and 0.6. There is a decrease in the density of incorporation of new cell wall material after invagination starts, as indicated by side-wall shading.

tion of the stress on the remaining bonds. By a continuous series of cytoplasm increases, surface stresses, enzymatic cuts and volume increases, one gets an increased cell volume that precisely accommodates the increase in cell cytoplasm. This description recapitulates Koch's surface stress model (Koch, 1983).

An alternative proposal (Wientjes and Nanninga, 1989) suggests that the peptidoglycan of the cell "increased more or less exponentially during the division cycle", but constricting cells synthesized peptidoglycan faster than unconstricted cells of the same length. The evidence for this was the observation, from quantitative electron-microscopic autoradiography, of an apparent sudden increase in the rate of peptidoglycan synthesis in cells when constriction started. It was concluded that there was a sudden increase in peptidoglycan synthesis at the start of invagination. While the observed exponential synthesis is in accord with the pressure model of cell wall regulation described above (fig. 3), the difference between constricting cells and non-constricting cells contradicts the pressure model. An alternative explanation of this change in synthetic rate is that cells in a population have a diameter that varies around some mean. For a given length, a cell with a larger width will have a larger volume than a cell with a smaller width. This larger cell volume means that the cell has more cytoplasm and more surface area. If at a given length wider cells form constrictions and narrower cells do not form constrictions, *i.e.* if constrictions are formed based on the mass rather than the length of a cell, then constricted cells would have a greater rate of peptidoglycan synthesis. This is not precisely because the wider cells have a larger surface, but because the wider cells increase their mass at a faster absolute rate than narrower cells of the same length. My calculations indicate that a very small variation in cell diameter could explain the observed autoradiographic data (manuscript in preparation). Thus, the autoradiographic data on the rates of peptidoglycan synthesis (Wientjes and Nanninga, 1989) are consistent with the pressure model of wall synthesis (Cooper, 1988a). The finding of an exponential pattern of peptidoglycan synthesis during the division cycle (Wientjes and Nanninga, 1989) was based on experiments using synchronized cultures produced by elutriation. It has been proposed (Cooper, 1989b) that the synchrony approach is unable to measure the small deviations from exponential peptidoglycan synthesis that have been determined with the backwards-membrane elution method (see Cooper (1989b) for a complete analysis of this method and a comparison with synchronization). The published measurements of peptidoglycan synthesis rates using synchronized cultures (Wientjes and Nanninga, 1989) are, however, consistent with the pressure model of wall synthesis (Cooper, 1988a).

An inside-to-outside mode of peptidoglycan growth, similar to that for *Bacillus subtilis*, has been proposed for *E. coli* (Schwarz and Glauner, 1988; Glauner, Holtje and Schwarz, 1988). 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 apparent release or recycling of peptidoglycan in *S. typhimurium* (Cooper, 1988a) or in *E. coli* B/r (Cooper and Hsieh, 1988). At this time, the inside-to-outside mode of surface growth cannot be excluded. Koch (1990) has presented the arguments against such an inside-to-outside mechanism of peptidoglycan growth.

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. I suggest that 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 that bacterial peptidoglycan increases. The observed doubling in the rate of phospholipid synthesis (acronym DROPS) during the division cycle (Joseleau-Petit *et al.*, 1984, 1987) is proposed to be due to the synchronization procedure (Cooper, 1989b). A careful reading of Pierucci's (1979) data on phospholipid synthesis obtained with the membrane-elution method suggests that the rate of phospholipid synthesis is similar to peptidoglycan synthesis. Studies in my laboratory using the membrane elution method and the "ratio-of-rates" analysis fully support the suggestion that the rate of membrane synthesis is similar to that of peptidoglycan. Furthermore, cell proteins can be divided into two parts, those associated with cytoplasm and those associated with 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 pattern, that which is synthesized during the division cycle with constant rates of synthesis. Proteins are synthesized during the division cycle with a pattern that is 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.

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 timed. 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 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 side wall growth as uneventful extensions of 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 unmeasurable and 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 cessation of pole formation. A new cell surface structure, the periseptal annulus (MacAlister *et al.*, 1983), 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.

Regulation of growth by cytoplasm accumulation.

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. 2) because the initiation of new rounds of DNA replication occurs when the cell has a unit amount of cytoplasm per origin of DNA (Donachie, 1968). This is why cell size at initiation is proportional to the number of growing points in the cell. The cell "titrates" the amount of cytoplasm, or some specific molecule (Løbner-Olesen *et al.*, 1989) 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 (Cooper, 1970). The synthesis of DNA and cell 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. The deterministic model of Koch and Schaechter (1962) is a general statement of these same principles

Generalizations and caveats.

The proposal made here is, in essence, a simplification, and there may be exceptions to the rules. Let me analyse some arguments that might be made against this simplified approach.

If, at the instant DNA synthesis was initiated, there were a sudden reduction in the pool of deoxythymidine triphosphate (TTP, a precursor of DNA), one might expect a relaxation of repression of the enzymes producing this molecule. A surge in the synthesis of these enzymes might be anticipated. As the concentration of TTP returns to normal, repression reduces the rate of enzyme synthesis. Even if such a synthetic pattern were too small to be experimentally measured, it is conceivable that such a variation in cytoplasm synthesis does exist. Does this mean we should consider this wave of enzyme synthesis a cell-cycle event? I suggest that this change in synthetic rate is not significant for the division cycle. When such an increase in the synthesis of a cell's enzyme occurs, there is an infinitesimal decrease in the synthesis of all the other proteins in the cell. Some ribosomes are now transferred to the translation of RNA messages determining the synthesis of enzymes related to TTP synthesis. There is no change in the total rate of cytoplasm synthesis. Another source of variation in individual enzyme synthesis during the division cycle is the possibility that the rate of enzyme synthesis may be determined by gene dosage. If this were the case, then at the instant a gene doubled by replication, there would be a sudden change in the rate of synthesis of a particular protein. It is suggested here that any such variation (what Arthur Koch has suggested should be called the "fine structure" of cytoplasm synthesis) is unimportant with regard to the regulation of the division cycle.

A similar analysis applies to DNA replication. Parts of the genome with unusual concentrations of bases might produce a slowing of fork movement. Whether such a variation exists may be difficult to demonstrate Changes in the rate of replication have been noted in

the region of termination (DeMassey *et al.*, 1987; Hill *et al.*, 1987). The important point is that this variation in the rate of fork movement is unimportant to the division cycle. What is important is the total time between initiation and termination. Any minor variations or fine structure are subsumed into the total time for a round of replication.

Note that none of the variations in synthesis discussed here are related to the specific regulation or control of the division cycle. All the cell requires is the expansion of the total, undifferentiated cell cytoplasm. The caveat presented here is that while there may be events and variations that occur during cell growth, due in part to the non-exponential synthesis of the genome and cell wall, these are essentially minor with respect to the regulation of the bacterial division cycle.

Segregation of DNA.

There are two pieces of quantitative data that describe the non-random segregation of DNA in *E. coli*. When segregation was analysed using the membrane-elution method, non-random segregation was constant over a wide range of growth rates (Pierucci and Helmstetter, 1976). When cells were analysed by the "presegregation-methocel" method, segregation was found to be non-random at slow growth rates, becoming more random as the growth rate increased (Cooper and Weinberger, 1977; Cooper *et al.*, 1978). Although these two results seem contradictory, they are both accommodated by a model proposing that DNA segregates as though the new strands were attached to new wall material (Helmstetter and Leonard, 1987). The apparent discrepancy between the two methods is due to the fact that the membrane-elution method examines the non-randomness of segregation at a particular cell division, while the presegregation-methocel method measures segregation in the total cell population. The constancy of non-random segregation using the membrane-elution technique means that the shape of the cell is constant at all growth rates. A constant shape means that the fraction of the cell devoted to pole is constant at all growth rates. A similar conclusion was reached during an analysis of the overshoot of cell length during a shift-up (Cooper, 1989a).

The bacterial growth law during the division cycle.

Some believe 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 (Burdett and Kirkwood, 1983)? 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.

I have proposed that there is no simple mathematical growth law regulating or describing bacterial growth during the division cycle (Cooper, 1988c). Bacterial growth during the division cycle is the simple weighted sum of the biosynthetic processes that are described by the three categories proposed here. Since the cytoplasm is almost 80 % of the cell weight, 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. 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 mass.

The applicability of the *E. coli* model.

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* (Nathan *et al.*, 1982), and a stochastic mechanism (Smith and Martin, 1973) as well as a rate-controlled model (Hola and Riley, 1987) 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 can only 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.

To illustrate this point, examine a simple *Gedanken* experiment. Consider the analysis of a particular cell cycle with and without the *E. coli* system as a prototype. Assume that we have found a cell in which the C period, the time for a round of DNA replication, and the D period, the time between termination and cell division, are proportional to the interdivision time. The synthetic patterns expected for three growth rates are presented in figure 4. The C and D periods are two-thirds and one-third of the total interdivision time. In contrast to the patterns seen in figure 2, the patterns of DNA synthesis in cells with 20-, 30- and 60-min doubling times are identical. The size of the newborn cells does not change with growth rate. What would we have deduced had we discovered this cell before we had analysed *E. coli?* It would have been concluded either that initiation starts at a particular cell size, or the act of cell division initiates DNA replication. In this imaginary cell, we cannot distinguish between the two conclusions. Now examine this cell after the discovery of the rules governing the initiation of DNA synthesis in *E. coli*. It would now be concluded that this cell fits the general model for *E. coli*, but the rate of DNA replication and the segregation period, for reasons not yet

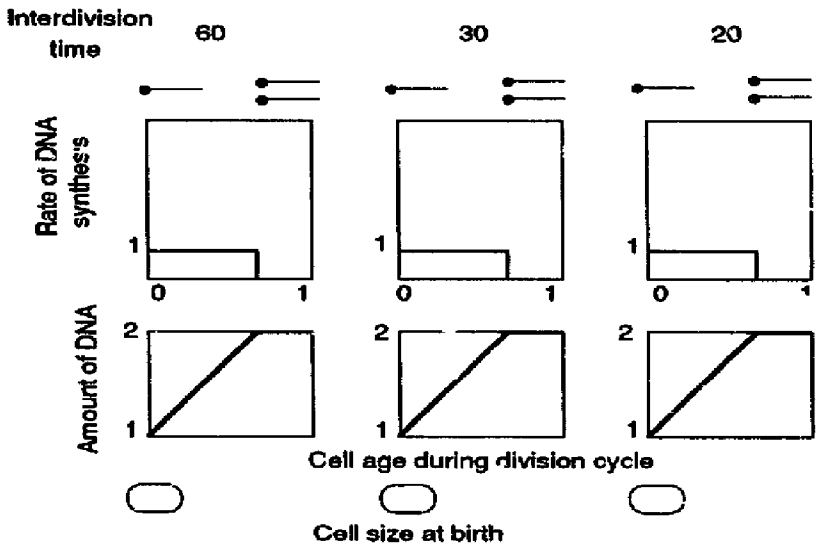

FIG. 4. — *DNA synthetic pattern if C and D periods are proportional to interdivision time*

This figure is similar to figure 2, except that C and D periods are drawn as two-thirds and one-third of the interdivision time In cells with 20-, 30- and 60-min interdivision times, the patterns of DNA synthesis are identical

understood, are not invariant with growth rate. The important point is that it was propitious that the C and D periods were constant in *E. coli*. This allowed the elimination of cell-cycle events, such as cell division, as causes of initiation of DNA replication. If the DNA synthetic pattern for *E. coli* were actually the one found in figure 4 rather than in figure 2, we would still be puzzling over the nature of the initiation rules in *E. coli*. Now that we have the rule, the *E. coli* rule, we cannot go back; we have the knowledge and it cannot be discarded. The only organisms that will be of interest in showing that new rules exist will be those that violate the rules laid down by our analysis of *E. coli*.

I have proposed that there is a unity of Cell Biology that is analogous to the unity of biochemistry (Cooper, 1981). Various phenomena which suggest 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 (Cooper, 1979; Cooper, 1982; Okuda and Cooper, 1989). The rules of the cell division cycle are the same for *Escherichia* and escargot. 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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