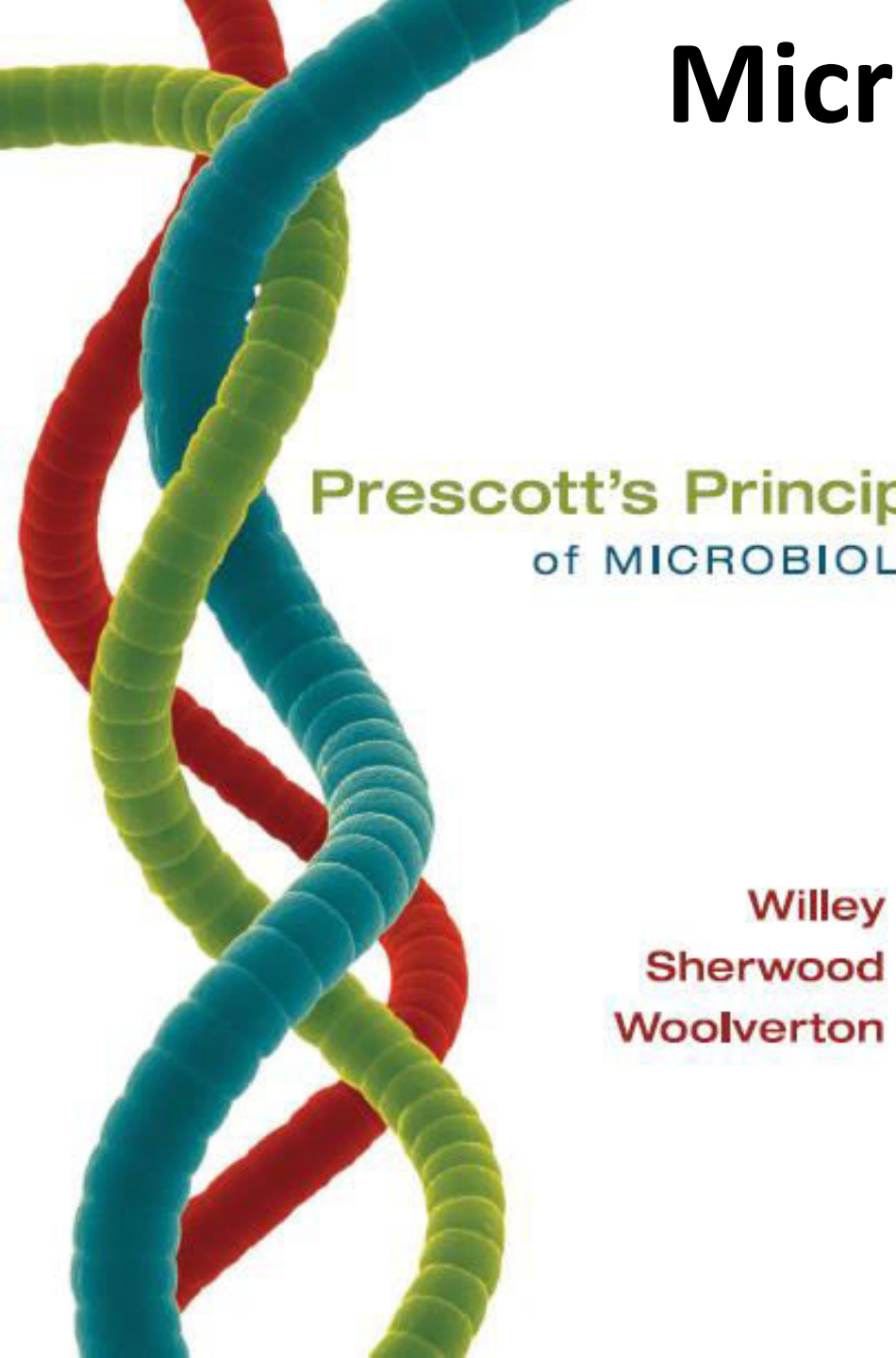
Microbial Growth Curve



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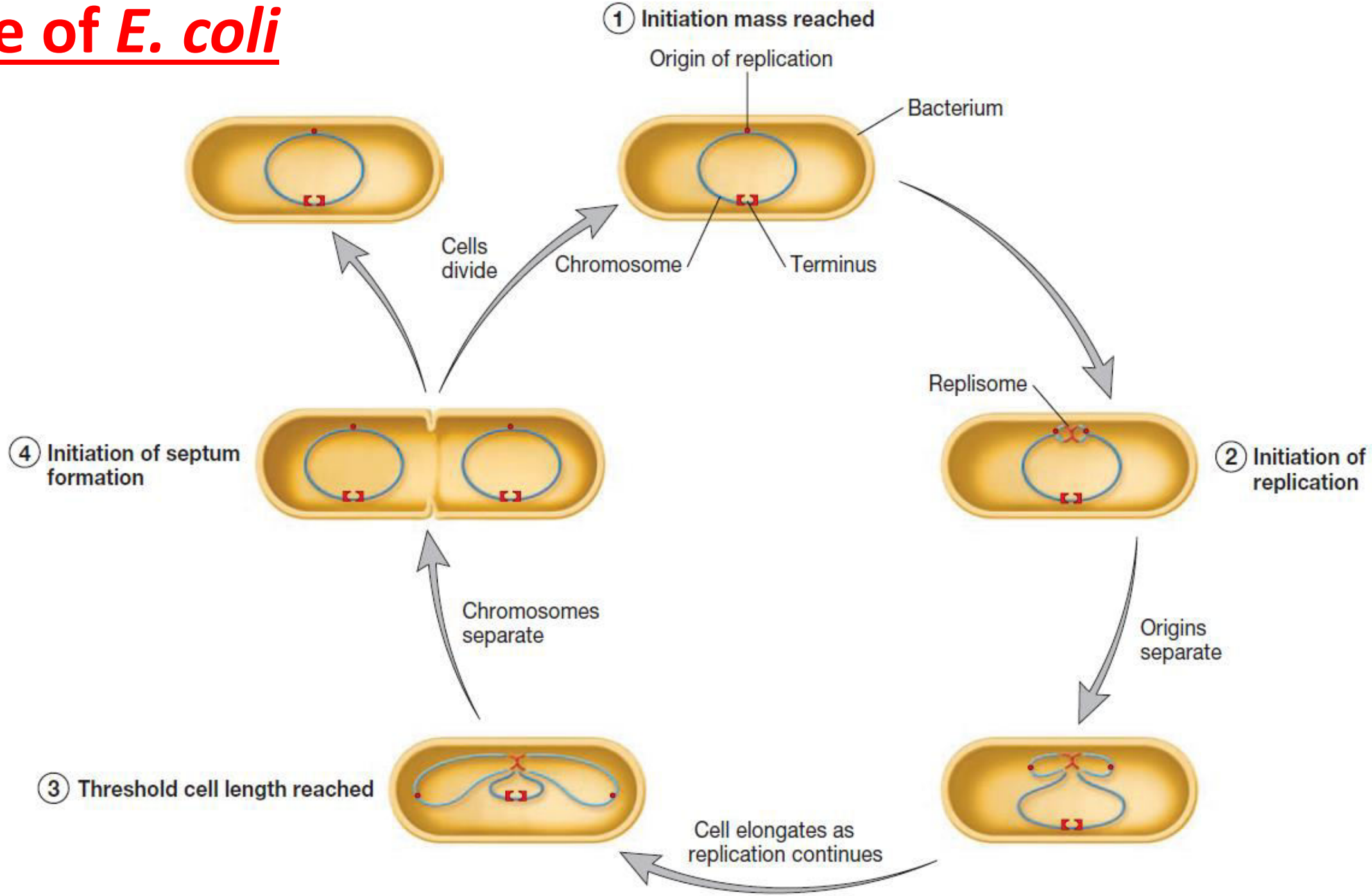
BACTERIAL CELL CYCLE

- Although some procaryotes reproduce by budding, fragmentation, and other means, most procaryotes reproduce by binary fission.

- Binary fission is a relatively simple type of cell division: the cell elongates, replicates its chromosome, and separates the newly formed DNA molecules so there is one chromosome in each half of the cell.

- Finally, a septum (cross wall) is formed at mid cell, dividing the parent cell into two progeny cells, each having its own chromosome and a complement of other cellular constituents.

Cell Cycle of *E. coli*



- However, a protein called **MreB**, which is **similar to eucaryotic actin**, seems to be involved in several processes, including **determining cell shape** and **chromosome movement**.

- MreB polymerizes to form a spiral around the inside periphery of the cell. One model suggests that the **origin of each newly replicated chromosome associates with MreB**, which then moves them to opposite poles of the cell.

- The notion that procaryotic chromosomes may be actively moved to the poles is further suggested by the fact that if MreB is mutated so that it can no longer hydrolyze ATP, its source of energy, chromosomes fail to segregate properly.

Cytokinesis

Septation is the process of forming a cross wall between two daughter cells. Cytokinesis , a term that has traditionally been used to describe the formation of two eucaryotic daughter cells, is now used to describe this process in procaryotes as well.
Septation is divided into several steps:

(1) selection of the site where the septum will be formed;

(2) **assembly of a specialized structure called the Z ring** , which divides the cell in two by constriction;

(3) **linkage of the Z ring to the plasma membrane** and **perhaps components of the cell wall**;

(4) assembly of the **cell wall-synthesizing machinery** (i.e., for synthesis of peptidoglycan and other cell wall constituents); and

(5) **constriction of the cell and septum formation**.

- The assembly of the Z ring is a critical step in septation, as it must be formed if subsequent steps are to occur.

- The **FtsZ** protein, a **tubulin homologue found in most bacteria** and many archaea, forms the Z ring. FtsZ, like tubulin, polymerizes to form filaments, which are thought to create the meshwork that constitutes the Z ring.

- Numerous studies show that the **Z ring is very dynamic**, with portions being exchanged constantly with newly formed, short FtsZ polymers from the cytosol.

- Another protein, called **MinCD**, is an **inhibitor of Z-ring** assembly.

- Like FtsZ, it is **very dynamic, oscillating its position** from one end of the cell to the other, forcing Z-ring formation only at the center of the cell.

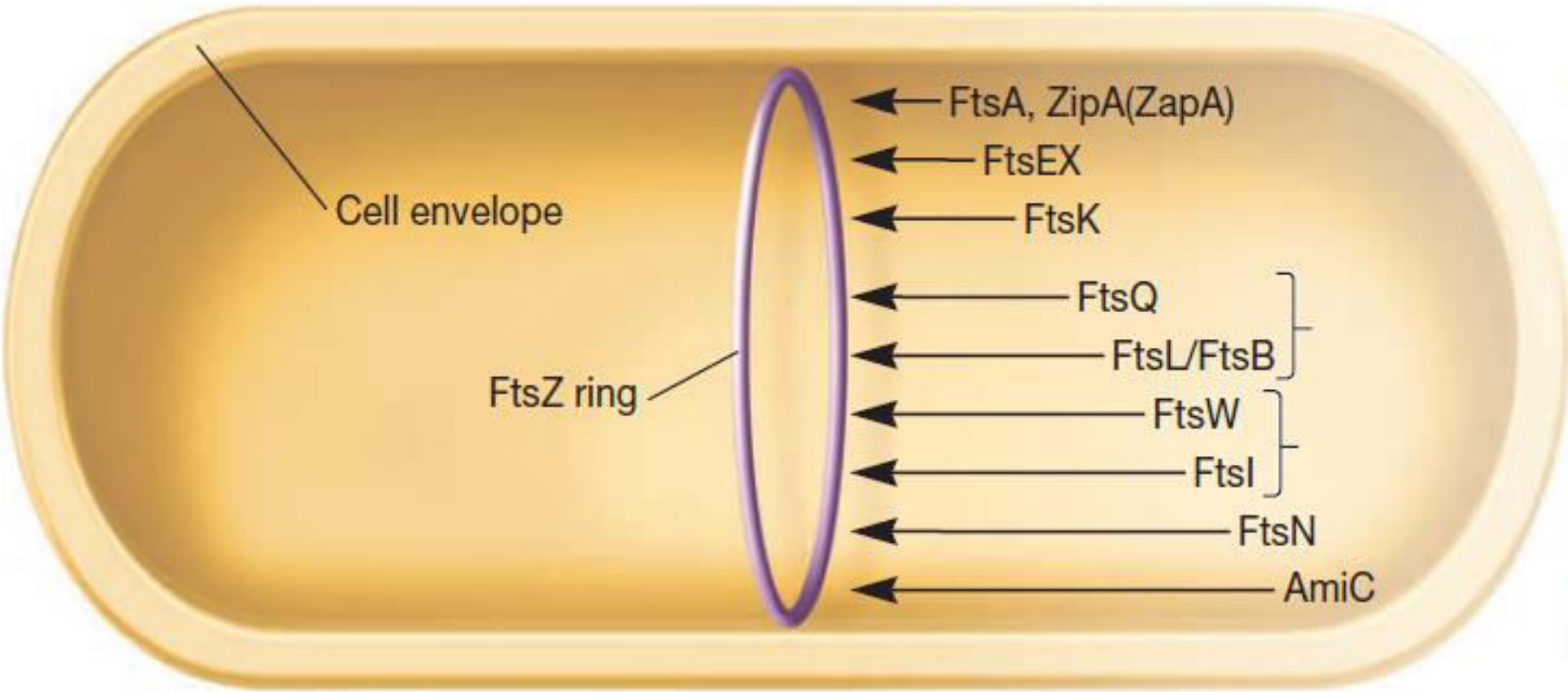
- First one or more anchoring proteins link the Z ring to the cell membrane. Then the cell wall-synthesizing machinery is assembled.

- The final steps in division involve **constriction of the cell by the Z ring**, accompanied by invagination of the cell membrane and synthesis of the septal wall.

- Several models for Z-ring function have been proposed.

- One model holds that the **FtsZ filaments are shortened by losing FtsZ subunits** (i.e., depolymerization) at sites where the Z ring is anchored to the plasma membrane.

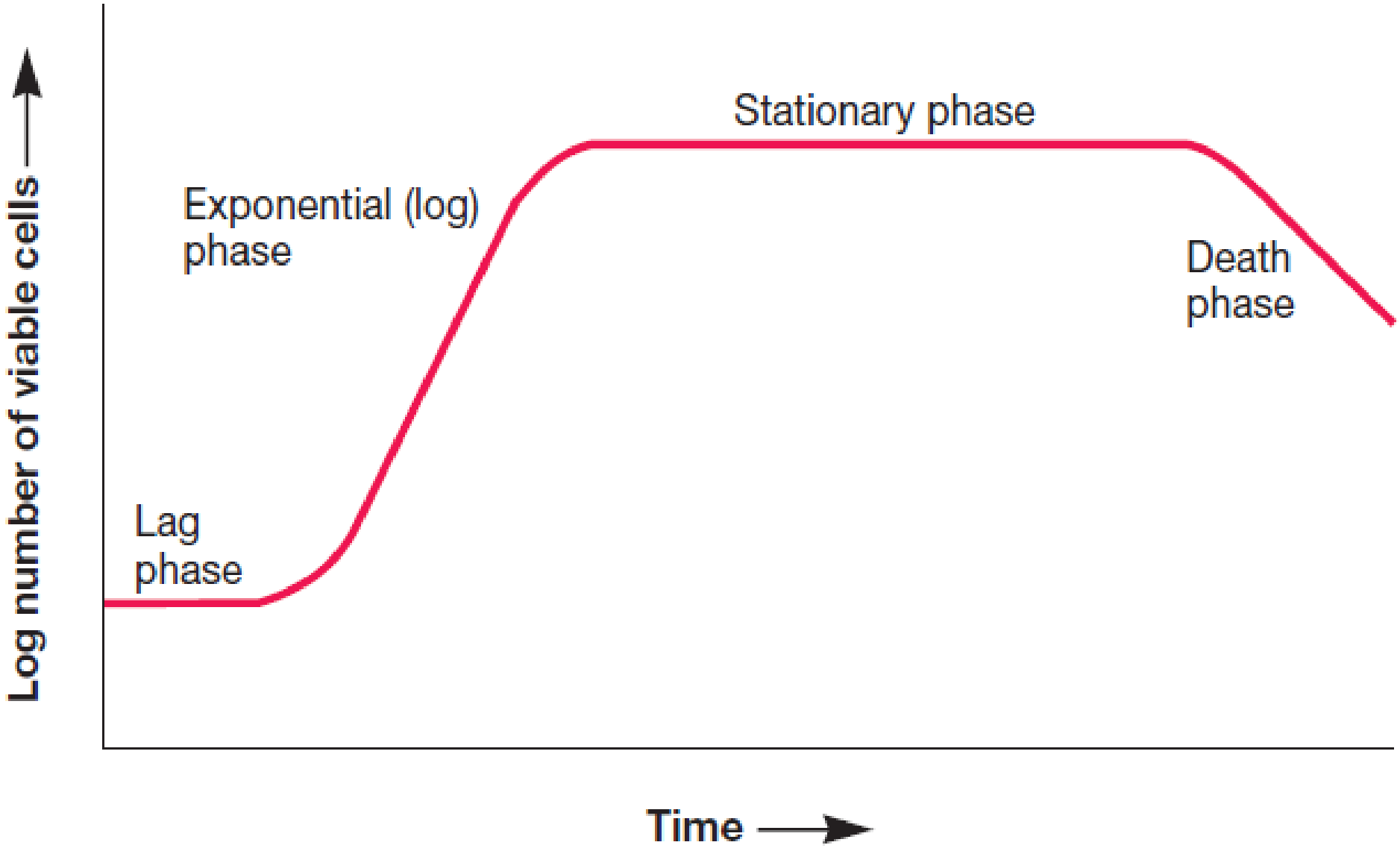
- This model is supported by the observation that Z rings of cells producing an excessive amount of FtsZ subunits fail to constrict the cell.



	Function
FtsA, ZipA(ZapA)	Anchors Z ring to plasma membrane
FtsEX	Unknown
FtsK	Coordinates septation with chromosome segregation
FtsQ	Unknown
FtsL/FtsB	
FtsW	Peptidoglycan synthesis
FtsI	
FtsN	Unknown
AmiC	Hydrolysis of peptidoglycan to separate daughter cells

GROWTH CURVE

- Binary fission and other cell division processes bring about an increase in the number of cells in a population.
- Population growth is studied by analyzing the growth curve of a microbial culture.
- When microorganisms are cultivated in liquid medium, they usually are grown in a batch culture that is, they are incubated in a closed culture vessel with a single batch of medium.
- Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase.
- The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time.
- The resulting curve has four distinct phase.


Log number of viable cells
Stationary phase
Exponential (log) phase
Death phase
Lag phase
Time

Lag Phase

- **When microorganisms are introduced into fresh culture medium**, **usually no immediate increase in cell number occurs**. This period is called the lag phase .

- However, **cells in the culture are synthesizing new components**. A lag phase can be necessary for a variety of reasons.

- The **cells may be old and depleted of ATP**, **essential cofactors, and ribosomes**; these must be synthesized before growth can begin.

- The **medium may be different from the one the microorganism was growing in previously.** Here new enzymes would be needed to use different nutrients.

- Possibly the **microorganisms have been injured and require time to recover**. Whatever the causes, eventually the **cells begin to replicate their DNA**, increase in mass, and finally divide.

Exponential Phase

- During the exponential (log) phase, microorganisms are **growing and dividing at the maximal rate** possible given their genetic potential, the nature of the medium, and the environmental conditions.

- Their **rate of growth is constant** during the exponential phase; that is, they are completing the cell cycle and **doubling in number at regular intervals**.

- The **population is most uniform** in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies.

- Exponential (logarithmic) growth is **balanced growth**. That is, all cellular constituents are manufactured at constant rates relative to each other.

- If nutrient levels or other environmental conditions change, **unbalanced growth** results.

- During unbalanced growth, the rates of synthesis of cell components vary relative to one another until a new balanced state is reached.

- Unbalanced growth is readily observed in two types of experiments:

  - **Shift-up**, where a culture is transferred from a nutritionally poor medium **to a richer one**.

  - **Shift-down**, where a culture is transferred from a rich medium **to a poor one**.

- In a **shift-up experiment**, **there is a lag while the cells first construct new ribosomes to enhance their capacity** for protein synthesis.

- In a **shift-down experiment**, there is a **lag in growth because cells need time to make the enzymes required for the biosynthesis of unavailable nutrients**.

- **Once the cells are able to grow again, balanced growth is resumed** and the **culture enters the exponential phase**.

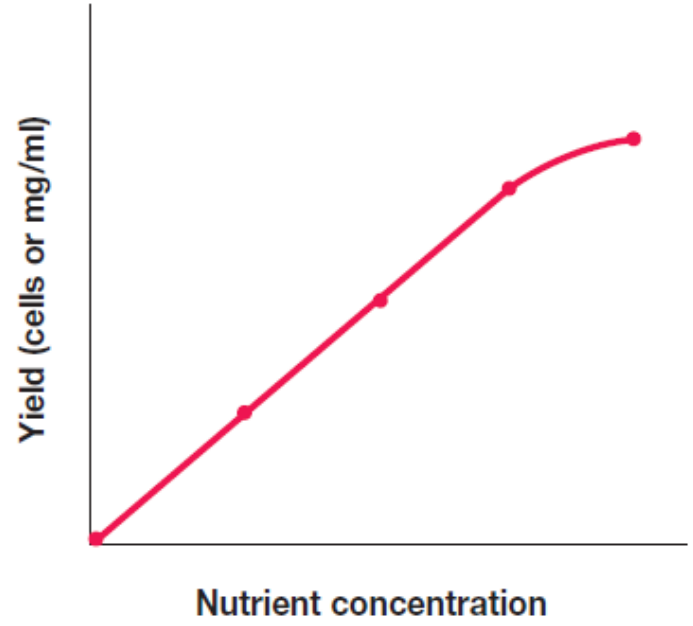
- These shift-up and shift-down experiments demonstrate that **microbial growth is under precise, coordinated control** and **responds quickly to changes in environmental conditions**.

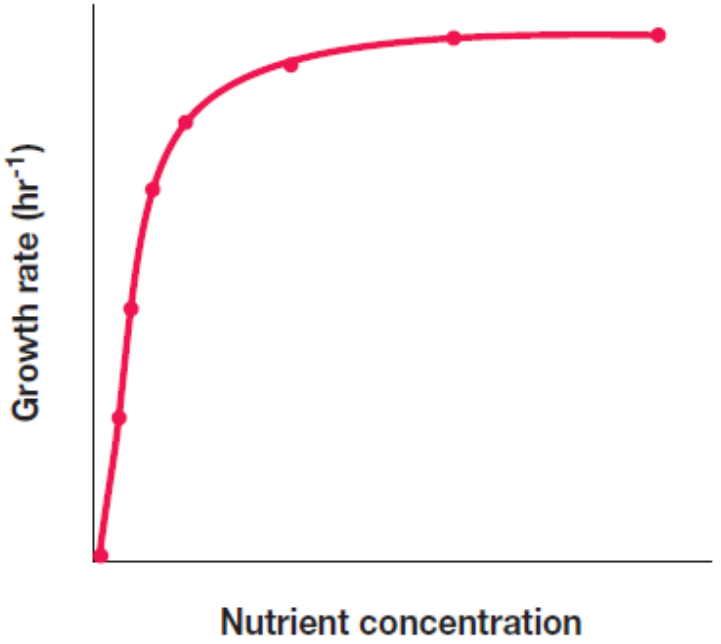
- **When microbial growth is limited by the low concentration of a required nutrient**, the **final net growth or yield of cells increases with the initial amount of the limiting nutrient present**.

- The **rate of growth also increases with nutrient concentration** but in a **hyperbolic manner** much like that seen with many enzymes.

- The **shape of the curve seems to reflect the rate of nutrient uptake** by microbial transport proteins.

- At sufficiently **high nutrient levels**, the **transport systems are saturated**, and the growth rate does not rise further with increasing nutrient concentration.





Stationary Phase

- In a closed system such as a **batch culture**, population growth eventually ceases and the growth curve becomes horizontal.
- This stationary phase usually is attained by **bacteria** at a population level of around **10^9 cells per ml**.
- Other microorganisms normally do not reach such high population densities.
- For instance, **protist** cultures often have maximum concentrations of about **10^6 cells per ml**.
- **Final population size depends on nutrient availability and other factors**, as well as the type of microorganism being cultured.
- In the **stationary phase, the total number of viable microorganisms remains constant**.

- This may result from a **balance between cell division and cell death**, or the population may simply **cease to divide but remain metabolically active**.

- Microbial populations enter the stationary phase for **several reasons**.

- One obvious factor is **nutrient limitation**; if an **essential nutrient is severely depleted, population growth will slow**.

- **Aerobic organisms** often are limited by **O_2 availability**. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O_2 concentration adequate for growth.

- The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way.

- Population growth also may cease due to the **accumulation of toxic waste products**.
- This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O_2).
- For example, **streptococci can produce so much lactic acid and other organic acids** from sugar fermentation that their **medium becomes acidic and growth is inhibited**.
- Finally, some evidence exists that growth may cease when a **critical population level is reached**.
- Thus entrance into the stationary phase may result from several factors operating in concert.

- As we have seen, bacteria in a batch culture **may enter stationary phase in response to starvation**. This probably occurs often in nature because many environments have low nutrient levels.

Procaryotes have evolved a number of strategies to survive starvation.

- Some bacteria respond with **obvious morphological changes** such as **endospore formation**, but many only decrease somewhat in overall size.

- This is often accompanied by **protoplast shrinkage and nucleoid condensation**.

- The more important changes during starvation are in gene expression and physiology. Starving bacteria frequently produce a variety of starvation proteins , which make the cell much more resistant to damage.

- Some increase peptidoglycan crosslinking and cell wall strength.

- The **Dps** (**DNA-binding protein from starved cells**) protein protects DNA.

- Proteins called **chaperone proteins** prevent protein denaturation and renature damaged proteins.

- Because of these and many other mechanisms, **starved cells become harder to kill** and **more resistant to starvation**, damaging temperature changes, oxidative and osmotic damage, and toxic chemicals such as chlorine.

- These changes are so effective that some bacteria can survive starvation for years. There is even evidence that

Senescence and Death

- For many years, the decline in viable cells following the stationary phase was described simply as the “death phase.”

- It was assumed that **detrimental environmental changes** such as **nutrient deprivation** and the buildup of **toxic wastes** caused irreparable harm and loss of viability.

- That is, **even when bacterial cells were transferred to fresh medium, no cellular growth was observed**.

- Because **loss of viability was often not accompanied by a loss in total cell number**, it was assumed that cells died but did not lyse.

- This view is currently under debate. There are **two alternative hypotheses**. Some microbiologists think starving cells that show an exponential decline in density have not **irreversibly lost their ability to reproduce**.

- Rather, they suggest that microbes are temporarily unable to grow, at least under the laboratory conditions used. This phenomenon, in which the cells are called **viable but nonculturable (VBNC)**, is thought to be the result of a genetic response triggered in starving, stationary phase cells.

- Just as some bacteria form endospores as a survival mechanism, it is argued that others are able to become dormant without changes in morphology. Once the appropriate conditions are available VBNC microbes resume growth.

- VBNC microorganisms could pose a public health threat, as many assays that test for food and drinking water safety are culture-based.

- The **second alternative** to a simple death phase is **programmed cell death**.

- In contrast to the VBNC hypothesis whereby cells are genetically programmed to survive, programmed cell death predicts that a fraction of the microbial population is genetically programmed to die after growth ceases.

- In this case, some cells die and the nutrients they leak enable the eventual growth of those cells in the population that did not initiate cell death.

- The dying cells are thus **“altruistic”** they sacrifice themselves for the benefit of the larger population.

Mathematics of Growth

Generation or doubling time

During the exponential phase, each microorganism is dividing at constant intervals. Thus the population doubles in number during a specific length of time called the **generation (doubling) time**.

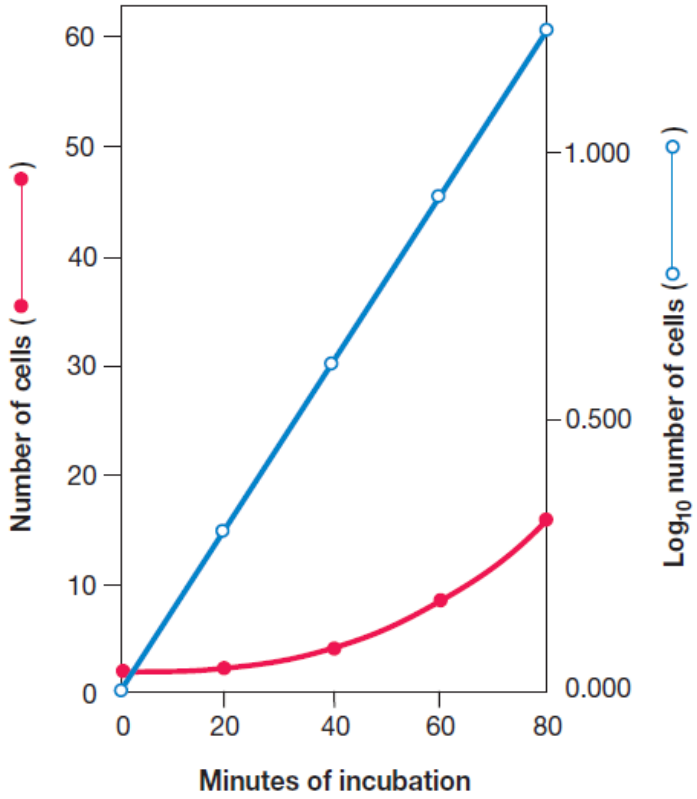
These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

$$N_t = N_0 \times 2^n$$



Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

Mean growth rate constant

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant (k)**. This is the **number of generations per unit time**, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

Mean generation (doubling) time

The time it takes a population to double in size-that is, the mean generation (doubling) time (g) can now be calculated. If the population doubles (t = g), then

$$N_t = 2N_0$$

Substitute $2N_0$ into the mean growth rate equation and solve for k.

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$