DNA transfer

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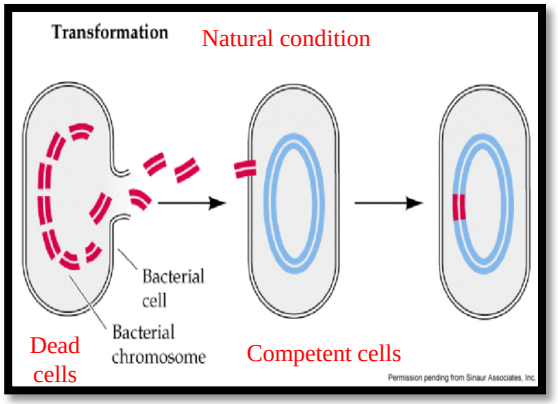
DNA transfer (نقل الدنا)

- Transfer of a recombinant DNA molecule to a cell is an essential step in DNA technology.
- **Natural condition :** Some bacterial cells, like those of the species Bacillus subtilis are able to take up DNA under physiological conditions **(natural transformation-التحول الطبيعي).**

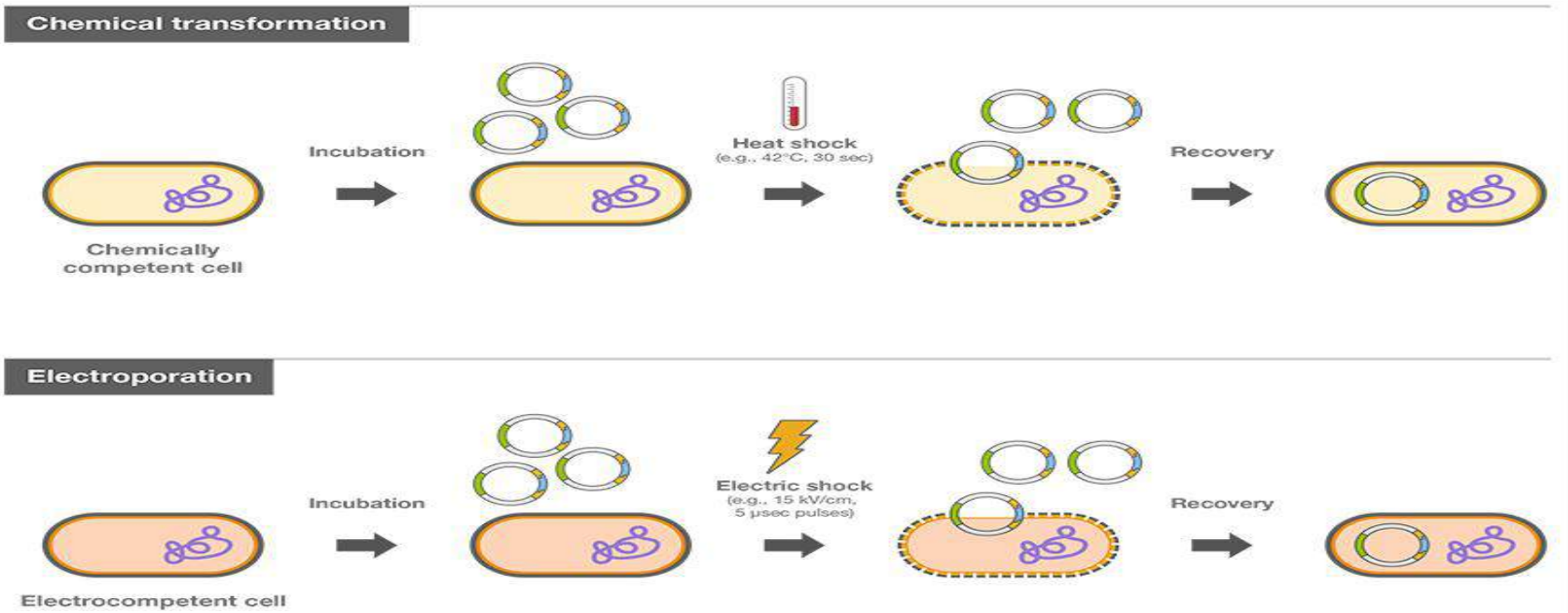
- **Artificial condition:** in most cases, microbial cells have to be forced by an unusual regimen to take up DNA **(non physiological condition)** by applying a **heat shock** to the host cells in the presence of high amounts of **Ca2+** ions. Or subjected to a vigorous **electrical discharge(تفريغ او تدفق كهربائي)** .

- Under those artificial conditions the cell envelope is forced to open itself, after which DNA may enter through the "holes"(فجوات) that are created

DNA transfer



- Transfer of a recombinant DNA molecule to a cell is an essential step in DNA technology.
- This process is described as transformation. In molecular biology **Transformation** is genetic alteration (تغيير جيني)of a cell resulting from the direct uptake(قبط) , incorporation and expression of exogenous genetic material (exogenous DNA) (الدنا خارجي المنشأ) from its surroundings and taken up through the cell membrane.

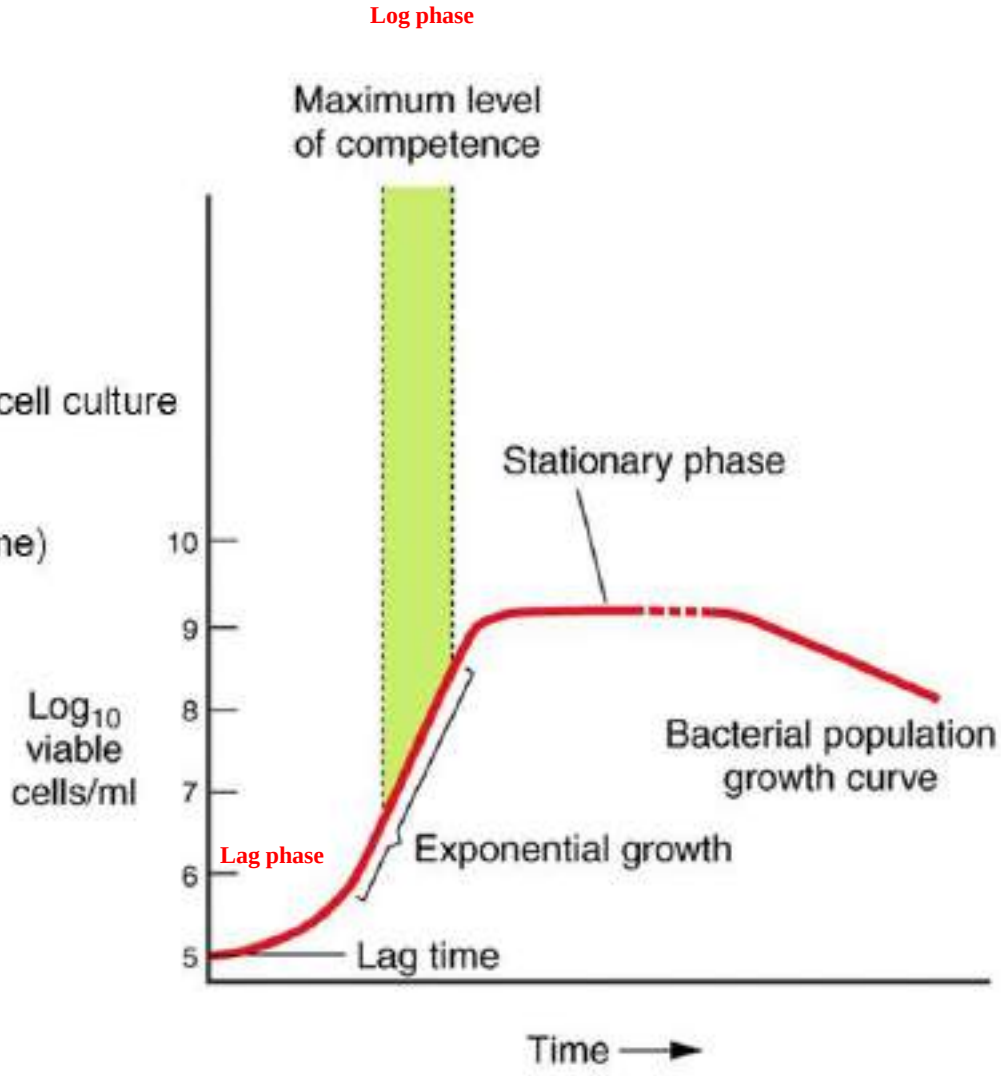


Competent bacterial cells (الخلايا البكتيرية المختصة)

Three properties of bacterial cell culture

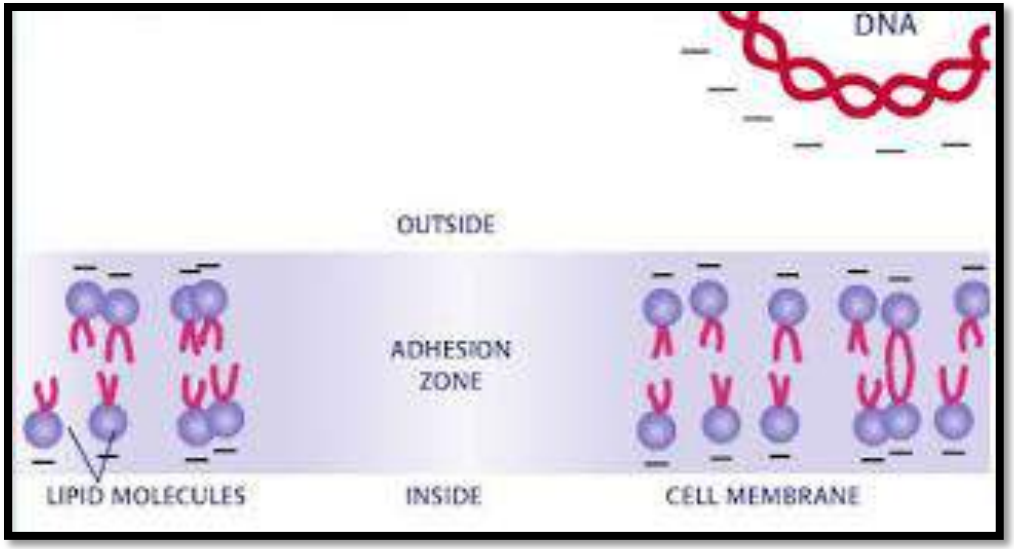
1. Phase of growth
2. Growth rate (generation time)
3. Cell concentration

Standart Curve!



Chemical transformation (التحول الكيميائى)

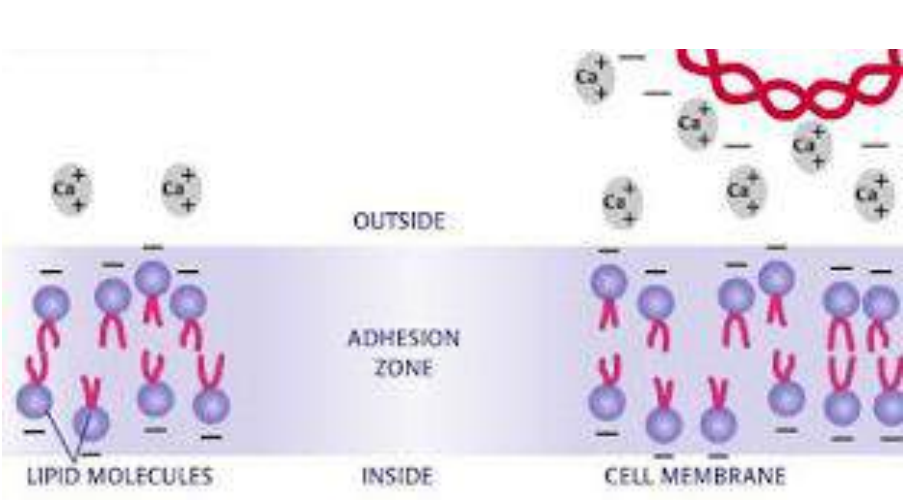
- Even though the adhesion zones (منطقة التماس أو اللإلتصاق) are physically large enough to admit small loop of DNA, the negatively charged phosphates on the DNA helix are repelled (ترفض) by those on the lipids.

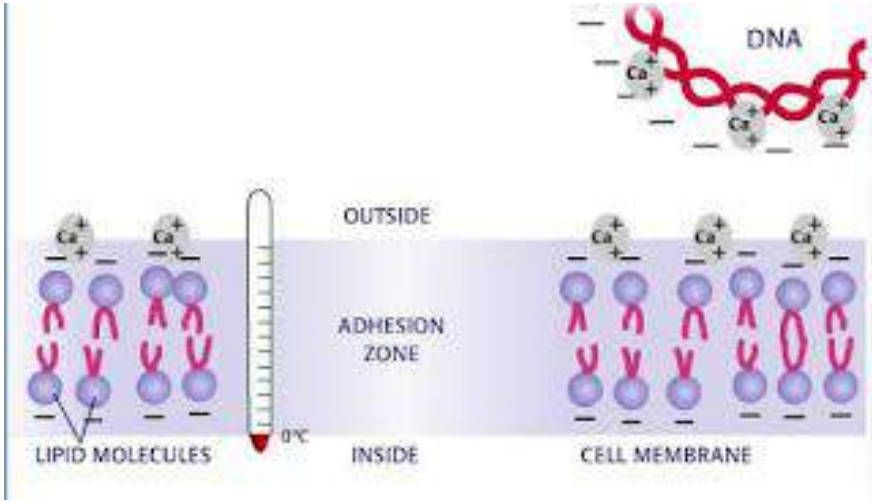


Chemical transformation

- Researcher use a combination of factors to make a bacterial cell capable of taking up new DNA (قادرة على قبط الدنا) . Theoretically, calcium (Ca2+) ions from added calcium chloride can interact with negative charges creating an electrostatically neutral situation (حالة معتدلة مستقرة كهربائيا).

- Lowering the temperature congeals (يحجر) the lipids membrane- stabilizing the negatively-charged phosphates and making them easier to shied.

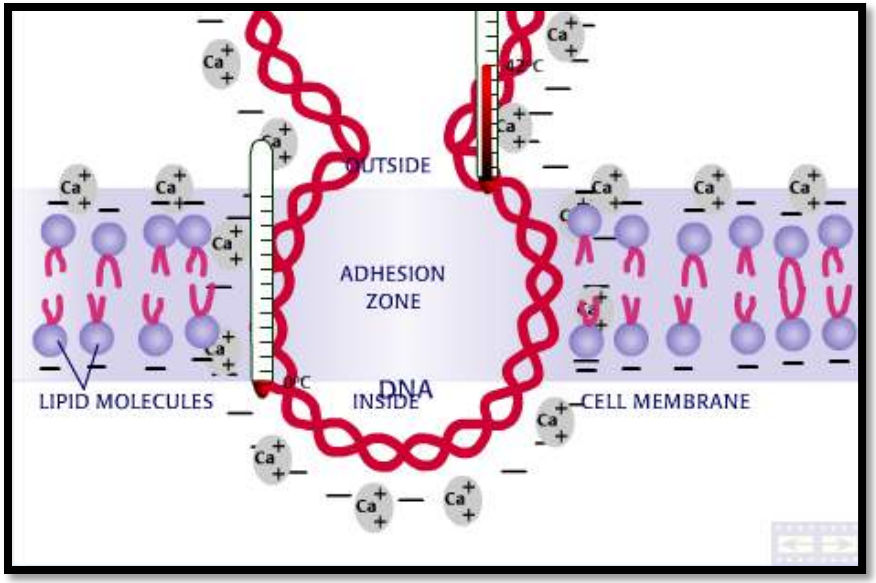




Chemical transformation

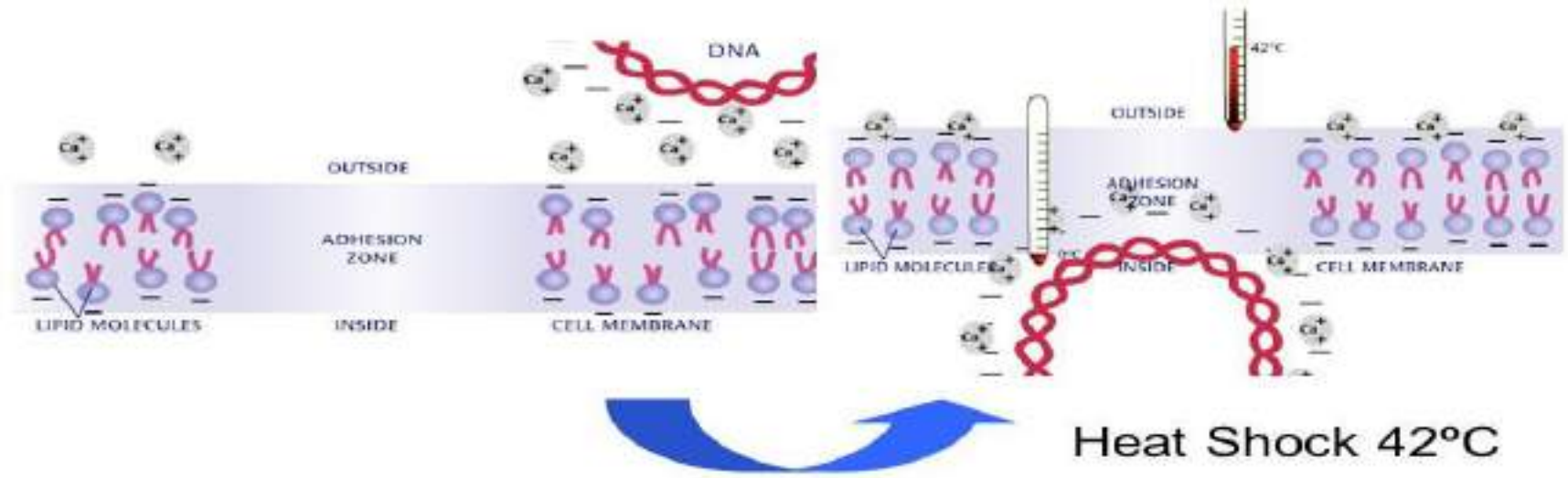
- A rapid rise in temperature or heat shock, then creates a temperature imbalance (عدم توازن حراري) on either side of the bacterial membrane, and sets up a current.

- With ionic shield (درع شاردي) in place, the DNA can then be swept through the adhesion zone.



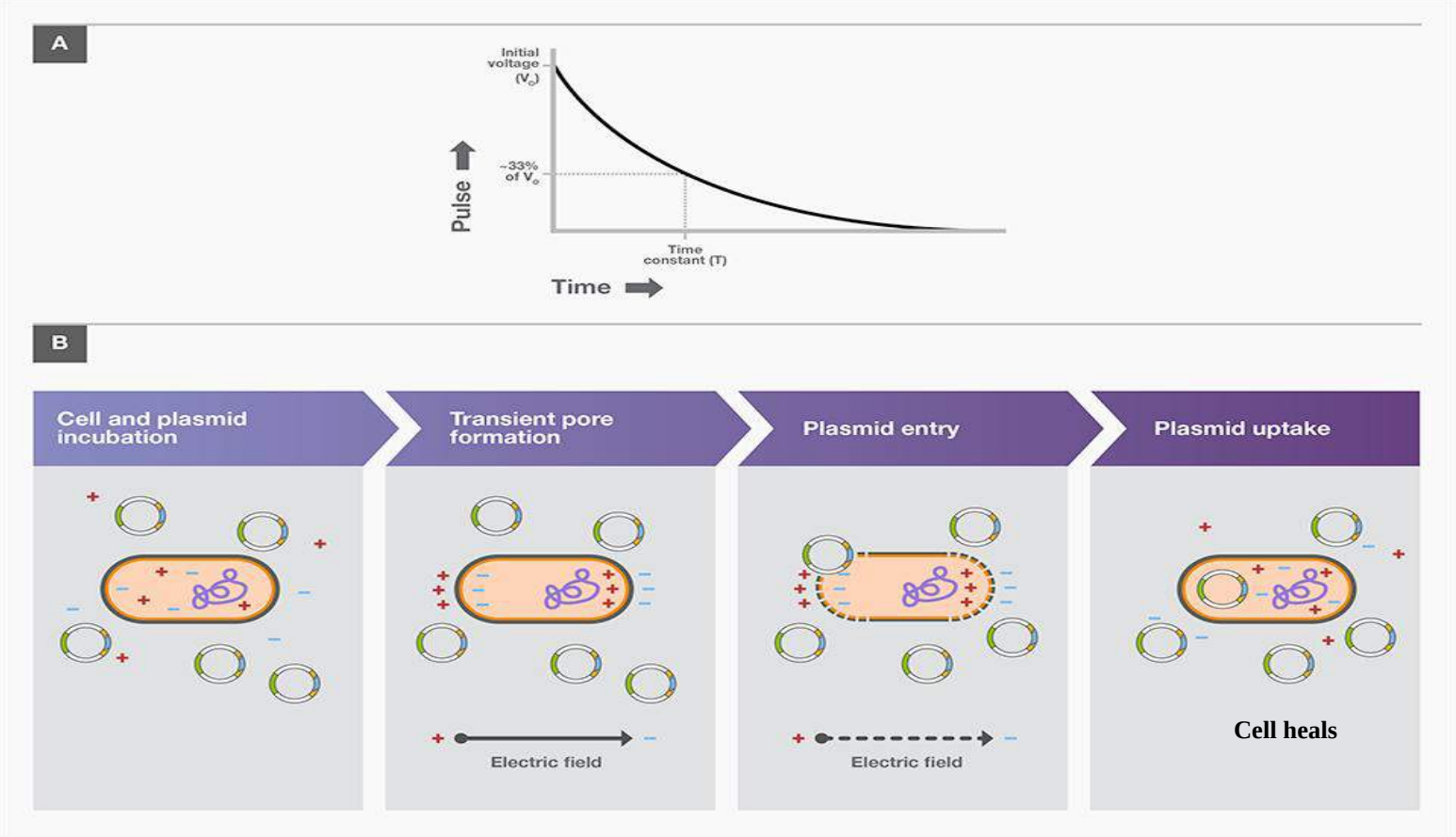
Chemical transformation

- The addition of calcium ions in CaCl2 solution shields the negatively charged phosphate groups so that the DNA can pass through the membrane when heat shocked.



Electrical transformation (تحو ل كهربائى)

- Another artificial method of **transformation** is electroporation, in which cells are shocked with an **electric** current, to create holes in the bacterial membrane

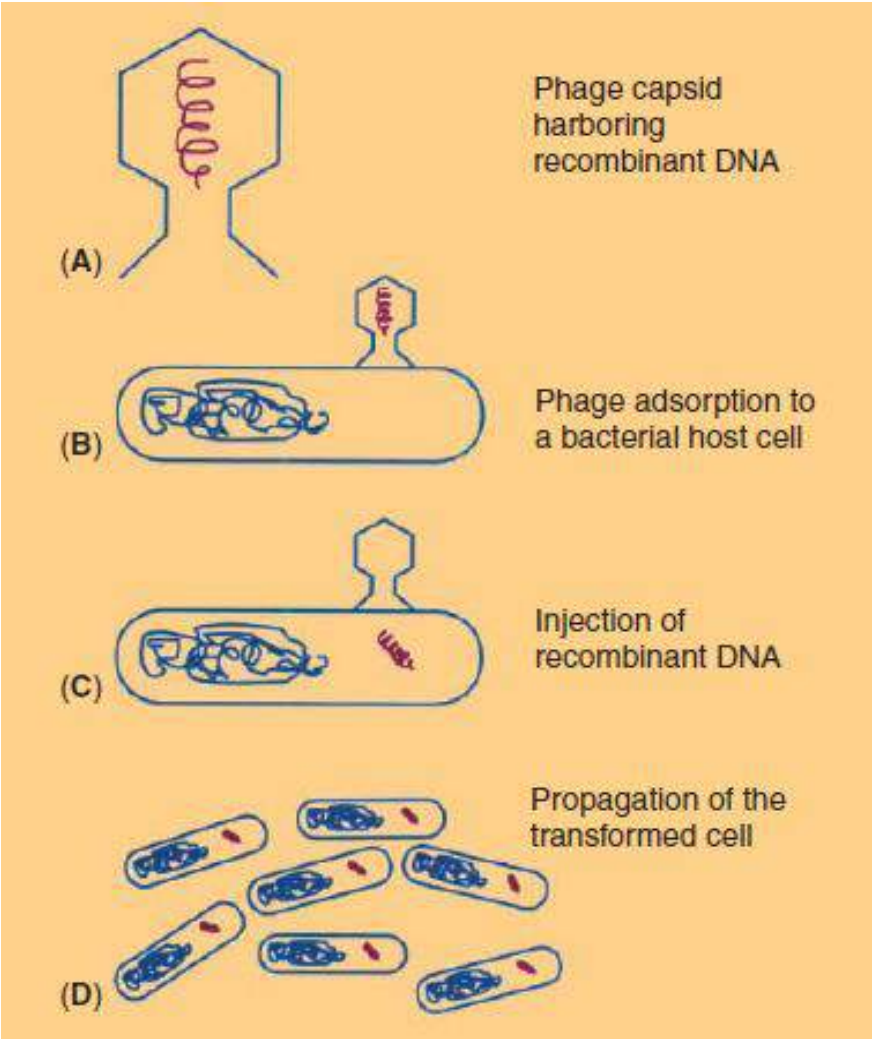


Electric field induces voltage across cell membrane

Other methods to transfer DNA by using Bacteriophages

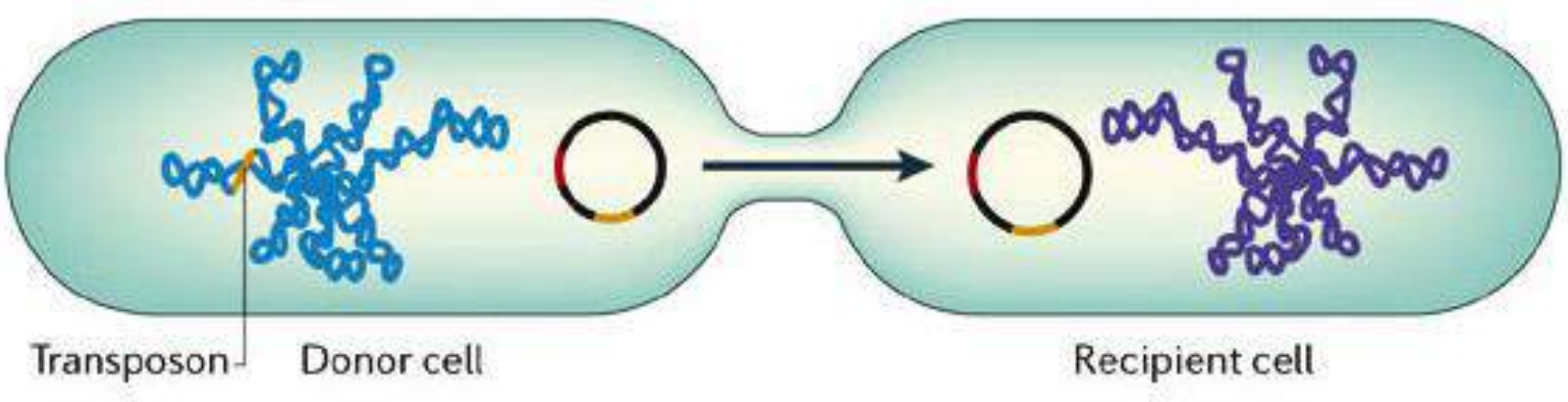
- Bacteriophage can be used as a mediator (وسيط) for DNA transfer by packaging the DNA in a bacteriophage capsid and then to mimic the normal bacteriophage infection procedure (عملية الخمج)

- Also viruses can be used as a vector (ناقل أو حامل) for the recombinant DNA technology, one may exploit natural virus infection processes to transfer DNA to an animal or a plant cell.



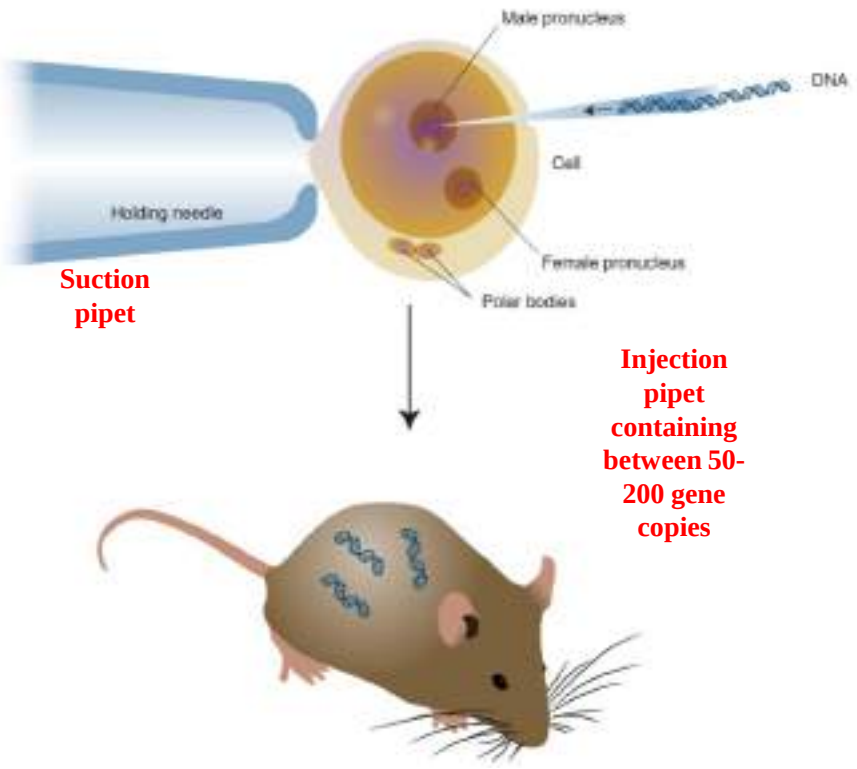
Other methods to transfer DNA by conjunction plasmid (بلاسميد إقتران)

- Transfer to bacterial cells can also be achieved by making use of conjugation.
- **Conjugation** is a process where DNA transfer takes place by cell–cell mating
- Needs a special class of plasmids is required, so called conjugative plasmids.
- If a cell with such a plasmid—the donor—meets a cell without such plasmid—the recipient—they may form together cell aggregates. as a consequence of a conjugative replication the plasmid transfer to the recipient cells.



Transfer DNA by Micro-surgery (الجراحة الدقيقة)

- Inject DNA (حقن الدنا)with a syringe into the nucleus of the cell.
- Is feasible due to the relative large dimensions of the animal cells compared to bacteria and is also applied to plant cells.
- The cell is brought on the tip of a thin glass tube and is fixed to the tube by suction (رشف أو مص)at the other end of the tube.
- By means of a micromanipulator (المعالج المجهري) a small syringe filled with DNA is directed to the nucleus of the fixed cell, and then the DNA is injected into the nucleus.



DNA transfer efficiency (فعالية نقل الدنا)

- The various techniques that are used to transfer DNA are generally not very efficient and may cause, as stated before, extensive killing of cells.

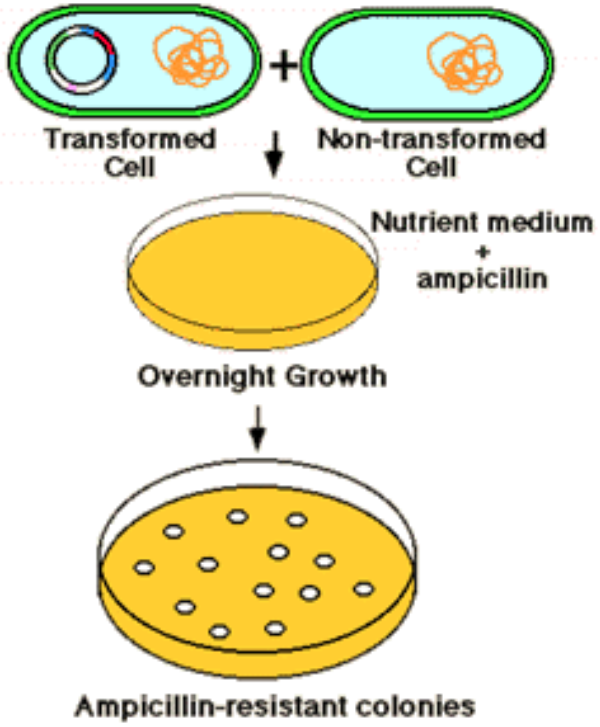
- in some cases the introduced DNA is subject to nuclease-mediated breakdown.

- while in animal or plant cells the introduced DNA does not always reach the nucleus, nor is it always integrated in a proper way.

- Therefore, selection techniques (تفنيات الإنتقاء) are highly desirable to find these rare cells.

DNA transfer efficiency

- Most selection techniques use a marker on the vector that codes for a selective property.
- Markers (واصمات)which code for a resistance (مقاومة)towards a specific antibiotic substance are frequently used.
- The treated cells either microbial cells, plant cells or animal cells) in a medium containing the relevant antibiotic, where just the cells carry the DNA with resistance gene can be grow.
- An alternative selection method uses recipient cells with specific growth deficiencies and vectors carrying genes which overcome such deficiencies.



Cell culture (استنبات أو زرع الخلايا)

- Cell culture can be defined as the process of cultivating cells and tissue outside the body of an organism (*in vitro)* (في المختبر) in artificial environment which stimulates the *in vivo* (في الجسم الحي) condition such as temperature , nutrition and protection from microorganism.

- Biotechnology depends heavily on techniques to cultivate pro- and eukaryotic cells.

- What can be cultivated in small flasks in a research laboratory cannot always be cultivated efficiently on an industrial scale.

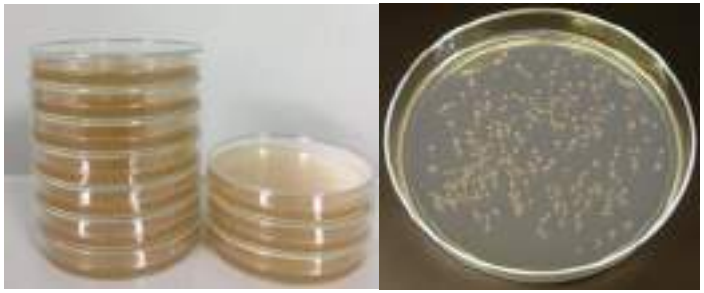
- Cultivation on an industrial level requires very sophisticated and delicate process technologies.

Cultivation of microbes (زراعة الميكروبات)

- In general, microbes can be cultivated either in vessels or tanks filled with an appropriate liquid growth medium or on plates containing a growth medium solidified with agar.

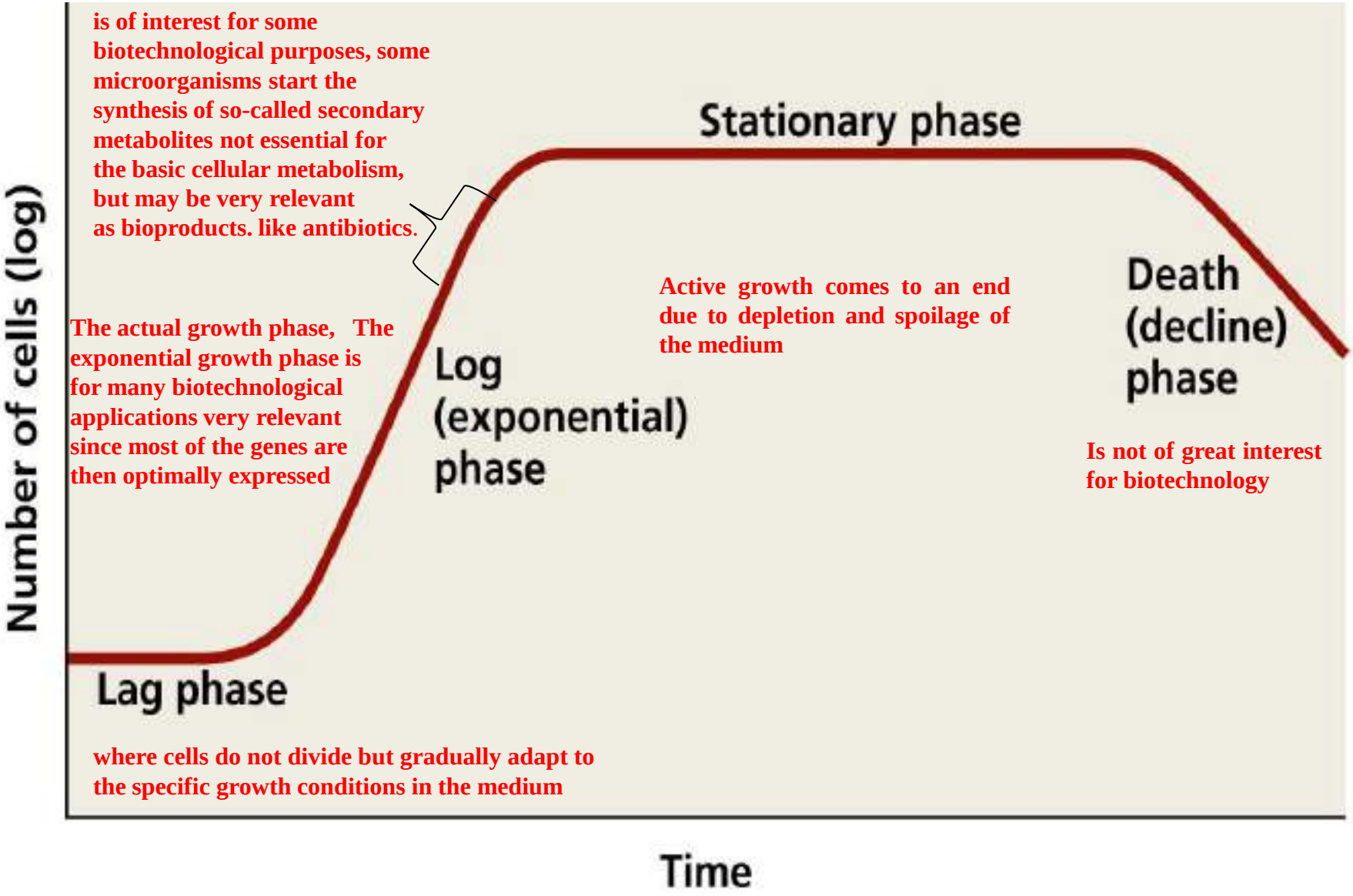
- The microbial growth in the medium will stop when the nutrition is depleted.

- there are **culture devices**, the continuos culture apparatus, which allow indefinite growth of the microorganism and called **continuous culture (استنبات مستمر)**.



- Most industrial biotechnology is based on culturing in tanks without a supply and overflow device. Such culture devices are called "**batch cultures."(استنبات كمية محددة)**

Growth curve



is of interest for some biotechnological purposes, some microorganisms start the synthesis of so-called secondary metabolites not essential for the basic cellular metabolism, but may be very relevant as bioproducts. like antibiotics.

The actual growth phase, The exponential growth phase is for many biotechnological applications very relevant since most of the genes are then optimally expressed

Active growth comes to an end due to depletion and spoilage of the medium

Is not of great interest for biotechnology

where cells do not divide but gradually adapt to the specific growth conditions in the medium

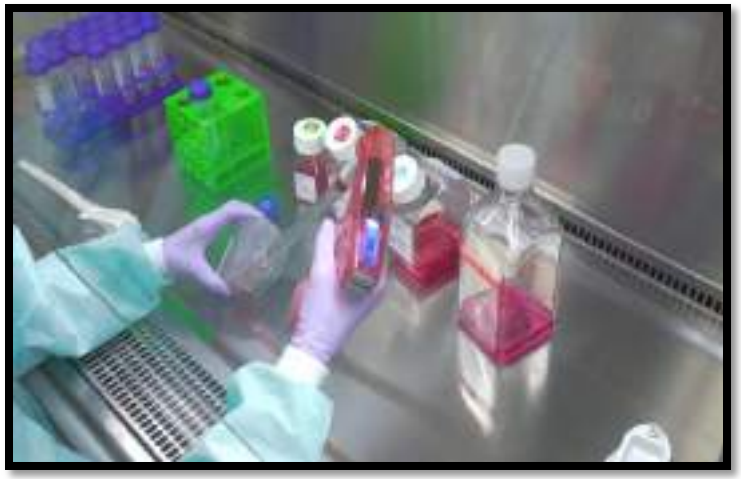
Microorganism growth curve (منحنى النمو) from point of view of Biotechnology

- **Lag phase (طور التاخر)** : lag phase, where cells do not divide but gradually adapt to the specific growth conditions in the medium precedes the phase where all cells start to divide.
- **Log phase or exponential phase (طور متسارع)** : The actual growth phase, is called the logarithmic or exponential phase. The exponential growth phase is for many biotechnological applications very relevant since most of the genes are then optimally expressed.
- **Stage where the exponential growth is about to end:** is of interest for some biotechnological purposes, some microorganisms start the synthesis of so-called secondary metabolites, not essential for the basic cellular metabolism, like antibiotics.
- **Stationary phase (طورثابت) :** where active growth comes to an end due to depletion and spoilage of the medium..
- **Death phase :** After some time the stationary phase is followed by a phase where the bacteria die off. This stage is clearly not of great interest for biotechnology.

Handling (معالجة) the microorganism growth to achieve the maximum

- In biotechnology time is money and maximum cell yields are therefore required.
- keep the lag phase as short as possible and to postpone the onset of the stationary phase.
- **The first goal** is achieved by inoculating the tank with cells that, by proper preculturing, are optimally adapted to the medium in the tank.
- **The second goal** is achieved in various ways, adding fresh medium near the end of the exponential phase. This technique is called “fed batch culture.” pH, oxygen tension and temperature have to be chosen appropriately and should be controlled while cultivating.
- Infection with other microorganisms should be prevented.

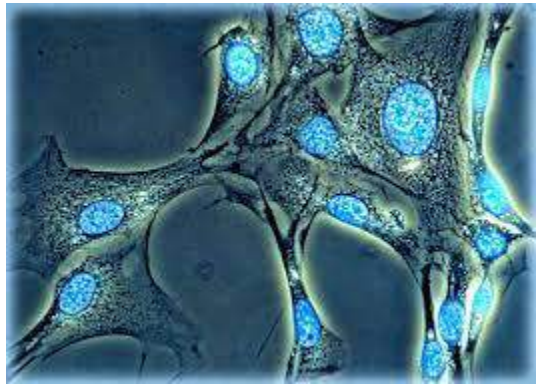
Animal cell culture (استنبات الخلايا الحيوانية)



- **The primary cell culture** (isolated out of a particular tissue after a protease (trypsin) treatment) is no useful for biotechnology.

- We use immortal cells, they may survive for months or even years, as long as they are diluted and recultured at frequent intervals (فترات متكرة) .

- Some cells of malignant origin (اصل ورمي) or originating from normal cells transformed by a virus like the Epstein Barr virus are immortal and grow to high cell densities but is not considered in the pharmaceutical biotechnology because may release the transforming virus which considered as contaminant for the pharmaceutical product.



- Most useful are non-malignant immortalized cell lines like 3T3 fibroblasts (خلايا ليفية) .

Thank you