212SM_L05b

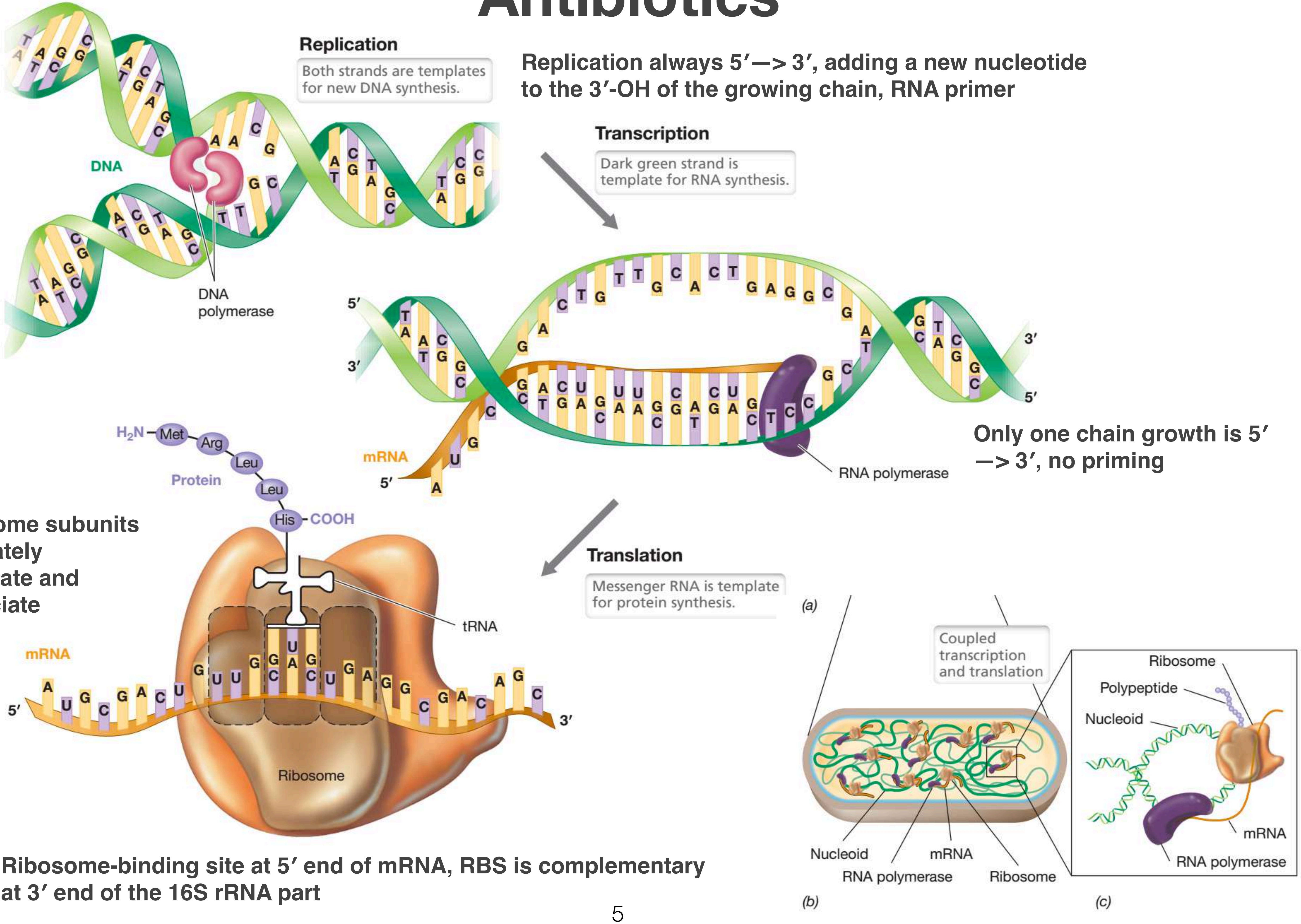
Microbial chemical weapons

THE ANTIBIOTICS

property	*E. coli*	budding yeast	mammalian (HeLa line)
cell volume	0.3–3 μm^3	30–100 μm^3	1000–10,000 μm^3
proteins per μm^3 cell volume	2–4 × 10^6		
mRNA per cell	10^3–10^4	10^4–10^5	10^5–10^6
proteins per cell	~10^6	~10^8	~10^{10}
mean diameter of protein	4–5 nm		
genome size	4.6 Mbp	12 Mbp	3.2 Gbp
number protein coding genes	4300	6600	21,000
regulator binding site length	10–20 bp	5–10 bp	
promoter length	~100 bp	~1000 bp	~10^4–10^5 bp
gene length	~1000 bp	~1000 bp	~10^4–10^6 bp (with introns)
concentration of one protein per cell	~1 nM	~10 pM	~0.1–1 pM
diffusion time of protein across cell ($D \approx 10$ μm^2/s)	~0.01 s	~0.2 s	~1–10 s
diffusion time of small molecule across cell ($D \approx 100$ μm^2/s)	~0.001 s	~0.03 s	~0.1–1 s
time to transcribe a gene	<1 min (80 nts/s)	~1 min	~30 min (incl. mRNA processing)
time to translate a protein	<1 min (20 aa/s)	~1 min	~30 min (incl. mRNA export)
typical mRNA lifetime	3 min	30 min	10 h
typical protein lifetime	1 h	0.3–3 h	10–100 h
minimal doubling time	20 min	1 h	20 h
ribosomes/cell	~10^4	~10^5	~10^6
transitions between protein states (active/inactive)	1–100 μs		
time scale for equilibrium binding of small molecule to protein (diffusion limited)	1–1000 ms (1 μM–1 nM affinity)		
time scale of transcription factor binding to DNA site	~1 s		
mutation rate	10^{-8}–10^{-10}/bp/replication		

Biology by the Numbers

Replication, Transcription, Translation & Antibiotics



Replication always 5′—> 3′, adding a new nucleotide to the 3′-OH of the growing chain, RNA primer

Only one chain growth is 5′ —> 3′, no priming

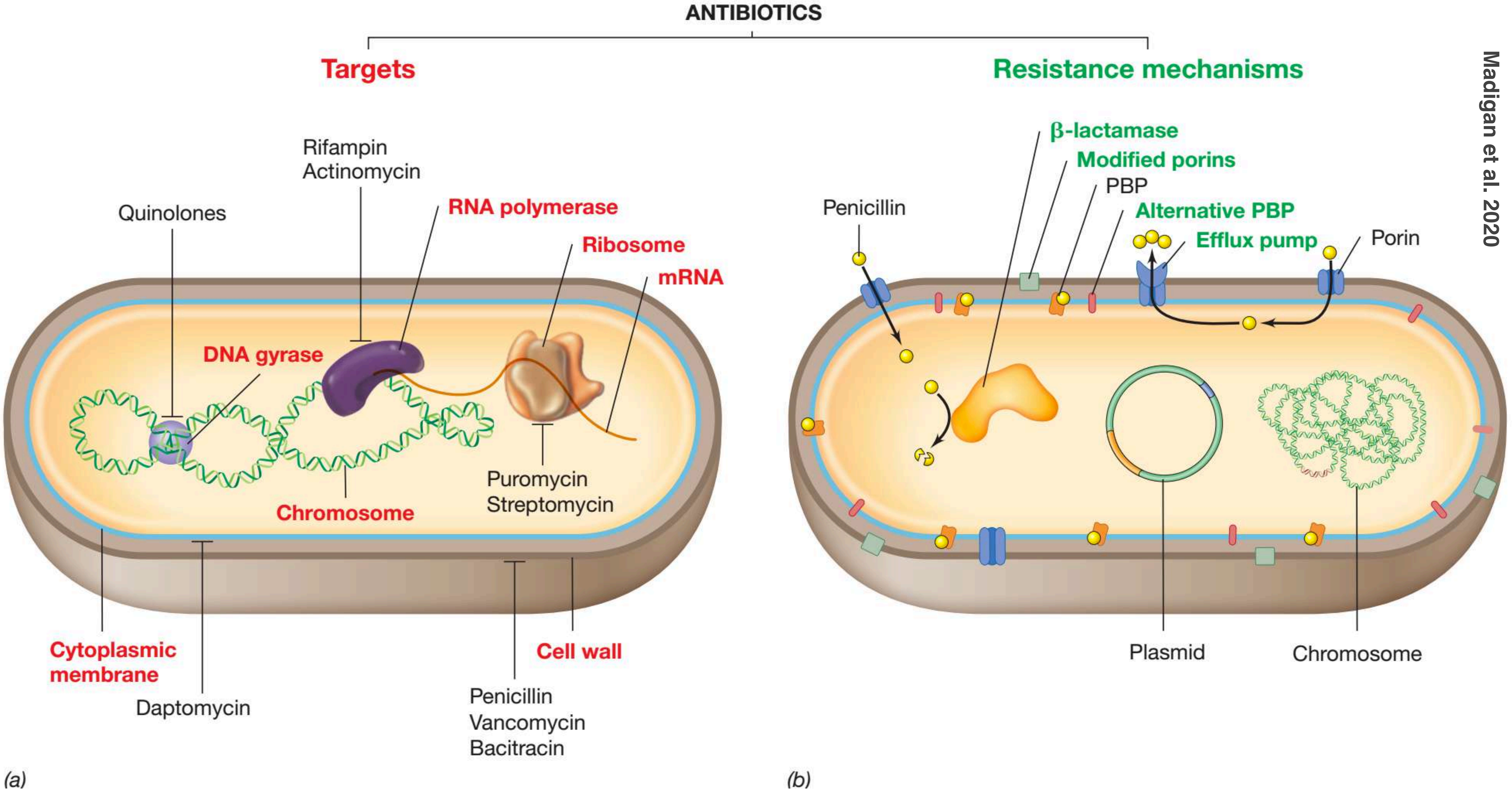
Ribosome subunits alternately associate and dissociate

Ribosome-binding site at 5′ end of mRNA, RBS is complementary at 3′ end of the 16S rRNA part

Madigan et al. 2020

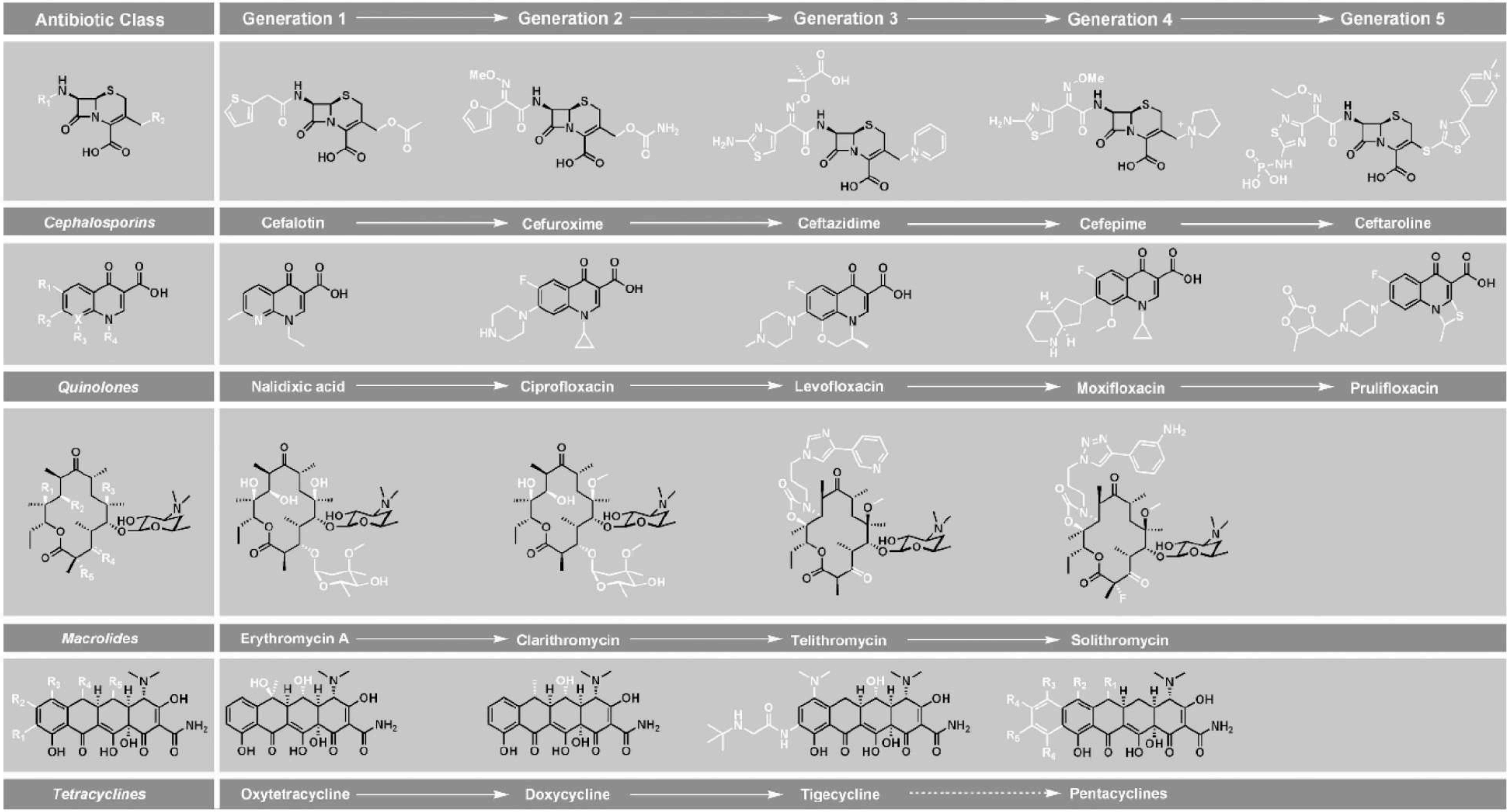
ANTIBIOTICS I

- **Antibiotics are antimicrobial agents naturally produced by microorganisms**, primarily certain bacteria and fungi to kill or inhibit bacterial growth



Madigan et al. 2020

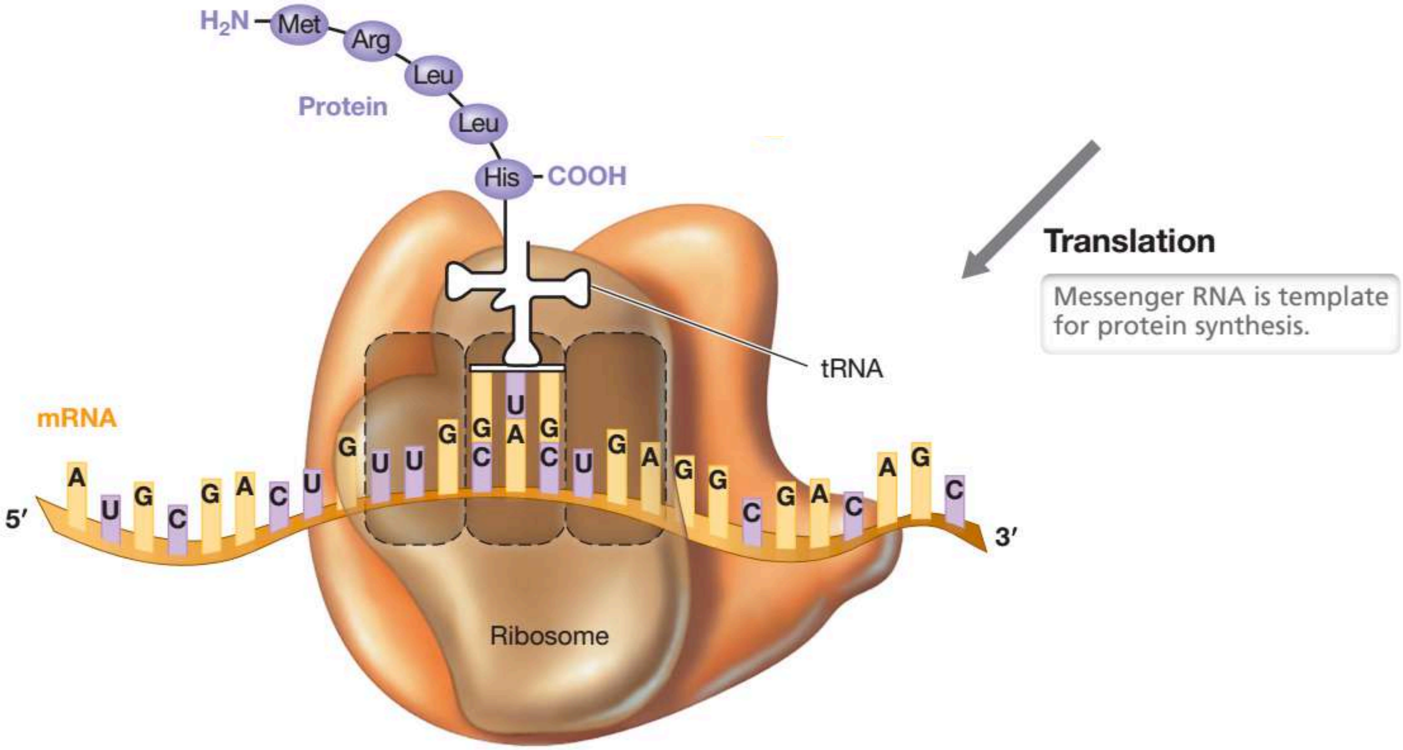
The shape of things



Walsh and Wencewicz, 2014

Translation & Antibiotics

- **Ribosome subunits alternately associate and dissociate**
- **Ribosome-binding site at 5′ end of mRNA, RBS is complementary at 3′ end of 16S rRNA**
- **Ribosomal RNA** plays role in ribosome subunit association, as well as in **positioning tRNAs on the ribosome (by 16S and 23S)**
- In addition to roles in mRNA alignment and translocation along the transcript, rRNA also catalyzes the actual formation of peptide bonds
- **Peptidyl transferase reaction occurs on the 50S subunit of the ribosome and is catalyzed solely by 23S rRNA**
- **rRNA also plays a role in translocation and interacts with the elongation factors and catalytic role in the process**

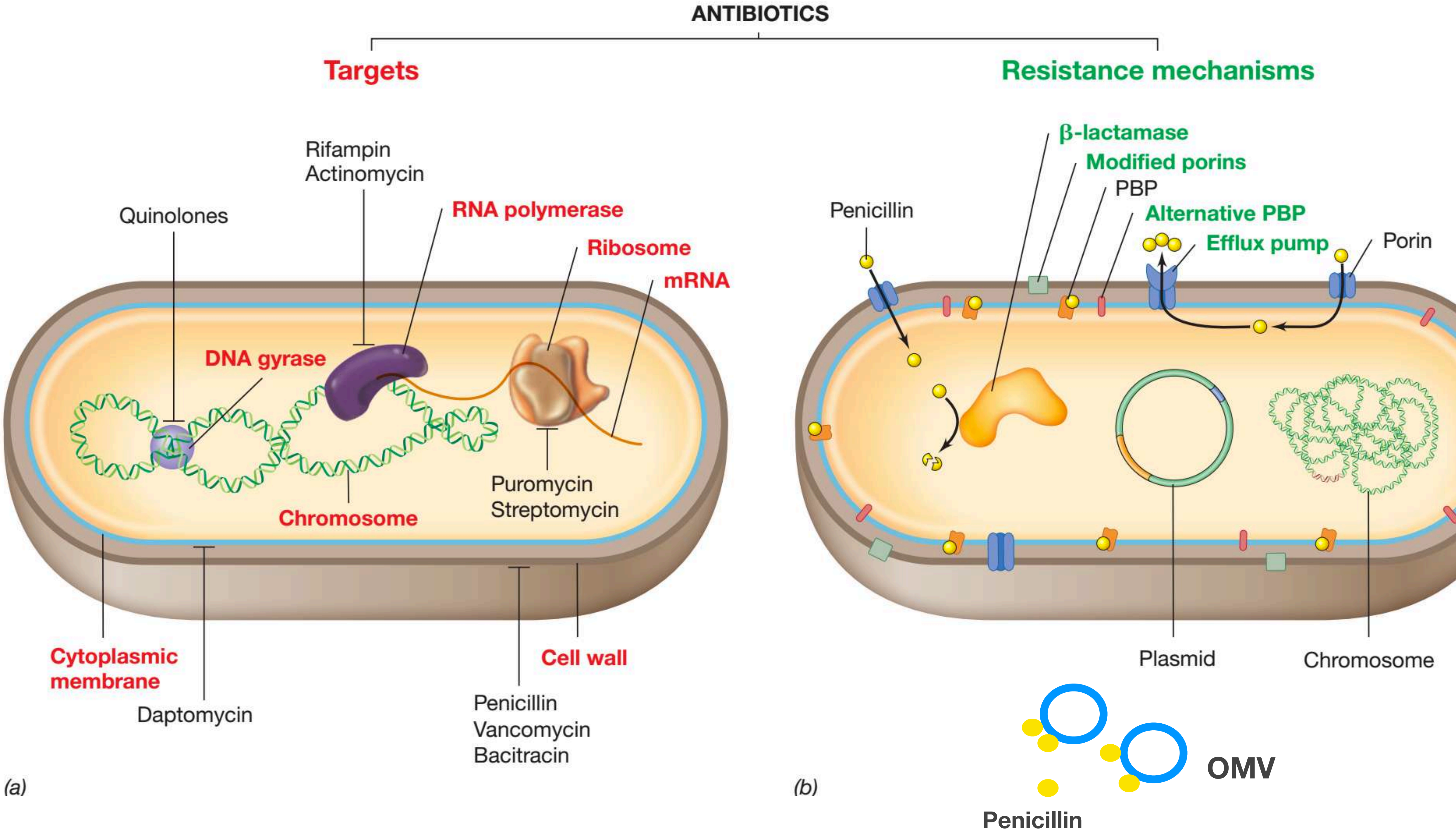


Madigan et al. 2020

ANTIBIOTICS II —> growth

- Antibiotics specifically target **enzymes** that catalyze **DNA replication**, **RNA synthesis**, and **translation**
- **Quinolones** such as ciprofloxacin target **DNA gyrase** in gram-negative bacteria and **topoisomerase** IV in gram-positive bacteria —> interfering with **DNA unwinding and replication**
- **Rifampin** and actinomycin **prevent RNA synthesis by either blocking the RNA polymerase** active site (rifampin) or **blocking RNA elongation** by binding to DNA major groove
- **Puromycin** contains a region that **mimics the 39 end of a tRNA**, and this structural mimicry results in specific **binding of the antibiotic to A site in the 70S ribosome** this induces **chain termination** and **inhibits protein synthesis**
- **Aminoglycoside** antibiotics such as streptomycin specifically **target 16S rRNA of 30S ribosome and result in ribosome misreading mRNAs**, thus leading to **error-filled proteins** that **accumulate** in cell and ultimately **inhibit growth**
- **Macrolide** antibiotic bind the bacterial **50S ribosomal subunit causing cessation of bacterial protein synthesis (**erythromycin, roxithromycin, azithromycin and clarithromycin**);** lactone rings with sugars

ANTIBIOTICS III



Madigan et al. 2018

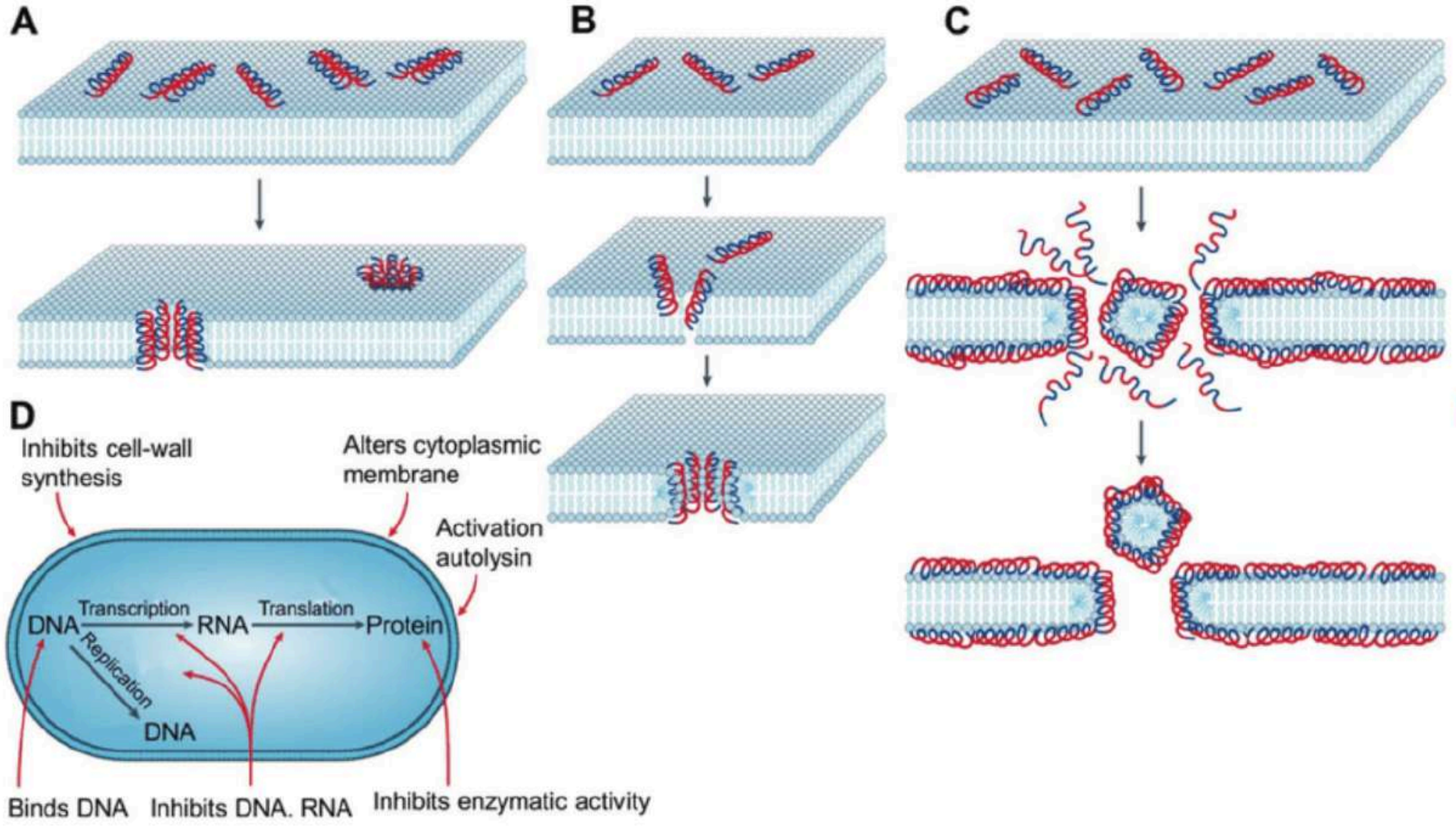
ANTIBIOTICS IV —> structures & metabolism

- **Daptomycin** is a lipopeptide produced by *Streptomyces* that specifically binds to phosphatidylglycerol residues of **bacterial cytoplasmic membrane** this leads to **pore formation and depolarization of membrane,** ultimately resulting in cell death
- Cell outer membrane: **polymyxins are cyclic peptides** whose long hydrophobic tails specifically target **LPS layer** and ultimately disrupt membrane structure, causing leakage and cell death
- **Synthesis of peptidoglycan** in bacteria such as **b-lactams penicillin, cephalosporin**, and their derivatives —> inhibit growth by **interfering with proteins that catalyze transpeptidation** (= **formation of cross-links between muramic acid residues that contribute to structural strength of peptidoglycan**)
- **Vancomycin** inhibits **peptidoglycan synthesis** in gram positive bacteria by **binding to pentapeptide of peptidoglycan precursors and preventing formation of peptide interbridges by transpeptidases**
- **Bacitracin** prevents **peptidoglycan synthesis by binding** to peptidoglycan precursor transport system and **preventing** new peptidoglycan precursors **from reaching site of peptidoglycan synthesis** —> autolysins continue to introduce small gaps in existing peptidoglycan a shortage of precursors to patch the gaps weakens cell wall and leads to cell lysis

Antimicrobial peptides

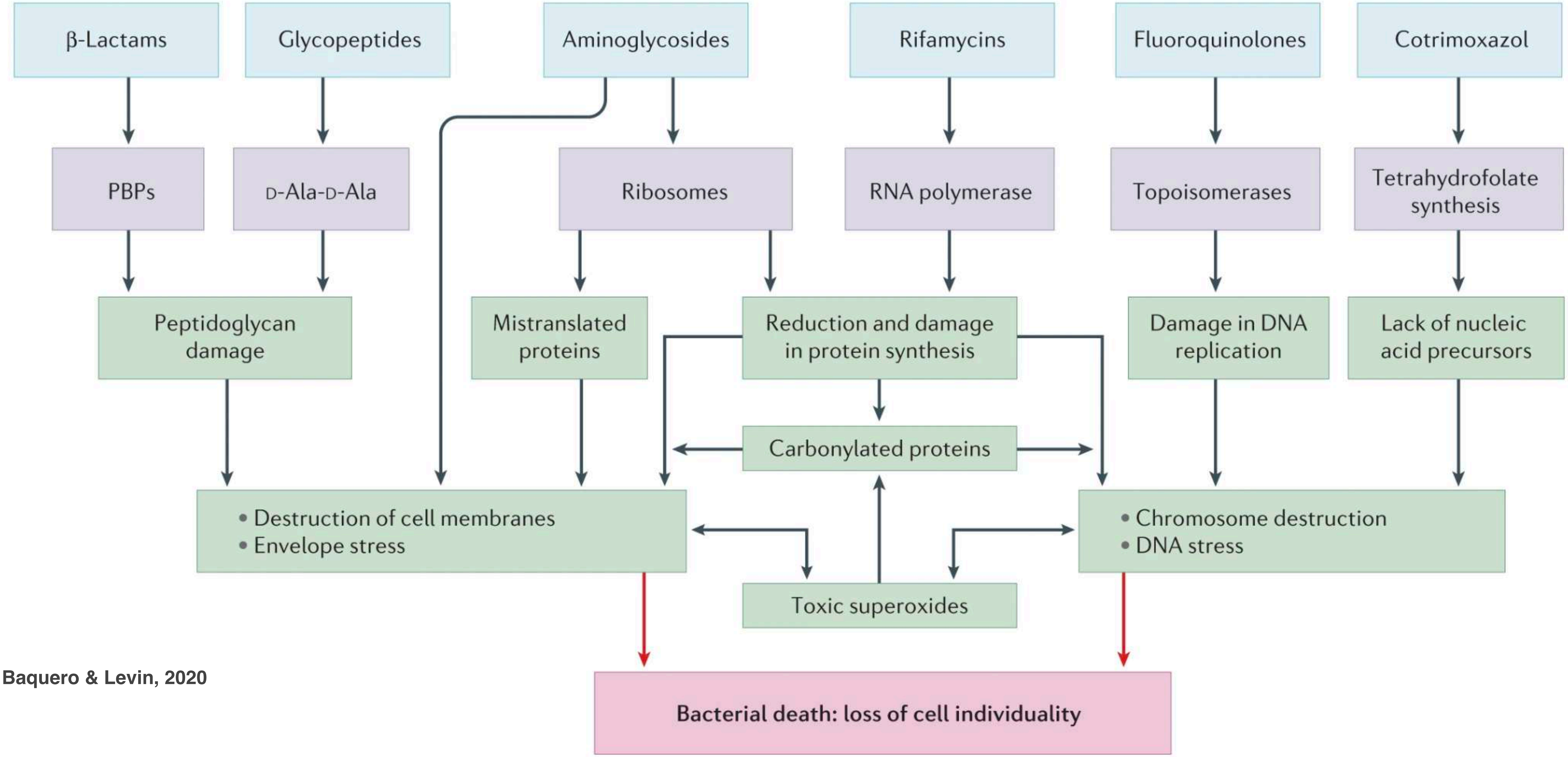
- **Antimicrobial peptides** (AMPs) are **oligopeptides** with a varying number (from five to over a hundred) of amino acids
- AMPs have a **broad spectrum** of targeted organisms ranging from viruses, bacteria, fungi to parasites
- Historically AMPs have also been referred to as cationic host defense peptides, anionic antimicrobial peptides/ proteins, cationic amphipathic peptides, cationic AMPs, host defense peptides, α-helical antimicrobial peptides
- AMPs kill cells by disrupting **membrane integrity** (via interaction with negatively charged cell membrane), by inhibiting proteins, DNA and RNA synthesis, or by interacting with certain intracellular targets

- **Cationic** AMPs attach to the negatively charged bacterial surface and membrane **by electrostatic interaction**, a prerequisite for AMP antimicrobial activity, which is often based on **pore formation** in the bacterial cytoplasmic membrane



Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria. A) Barrel-stave model, B) toroidal model, and C) carpet model of antimicrobial peptide-induced killing. D) Mode of action for intracellular antimicrobial peptide activity. Reproduced with permission. [138] Copyright 2005, Nature.

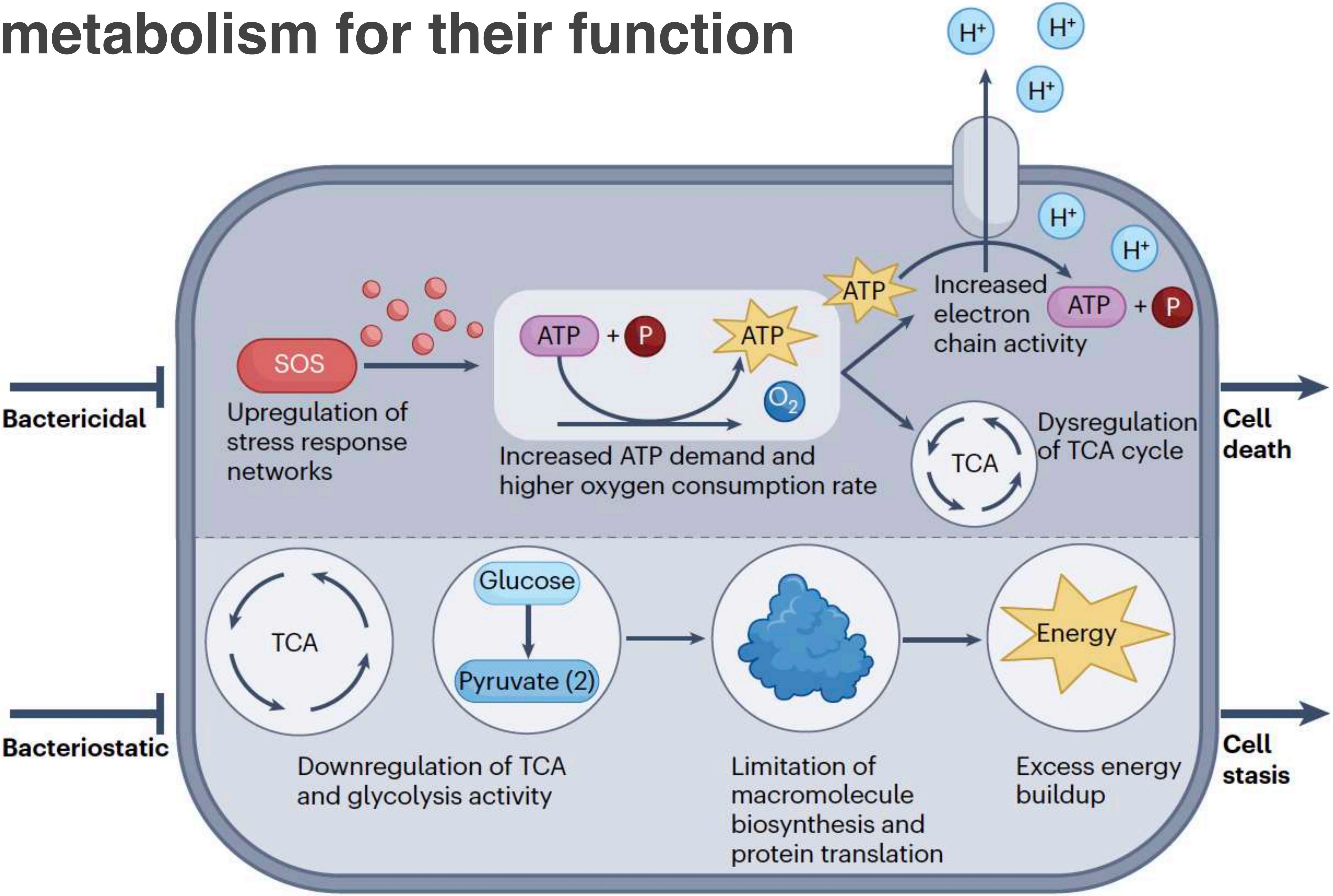
Successive steps in the process of bacterial killing by antibiotics from six families



Baquero & Levin, 2020

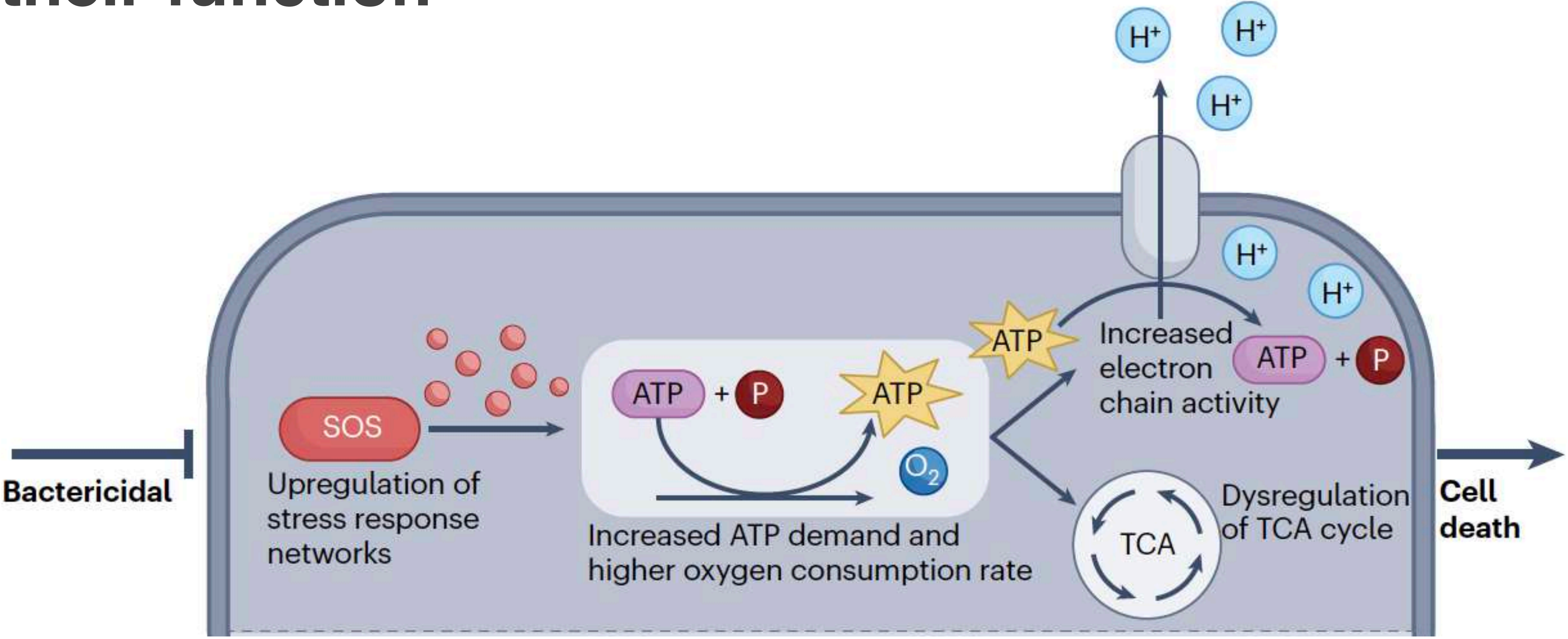
These drugs (blue) directly interact with their targets (purple), which results in structural damage and/or quantitative or qualitative deficiencies of essential cell components. These changes, in turn, lead to envelope stress, DNA damage and/or the production of an excess of reactive oxygen species, which further contribute to the destructuring of cell membranes and nucleic acids. The net effect of these different processes (green) is the ultimate mechanism responsible for the loss of the cell's individuality, its death (red).

Bactericidal and bacteriostatic antibiotics leverage metabolism for their function



Ahmad et al., 2025

Bactericidal antibiotics leverage metabolism for their function

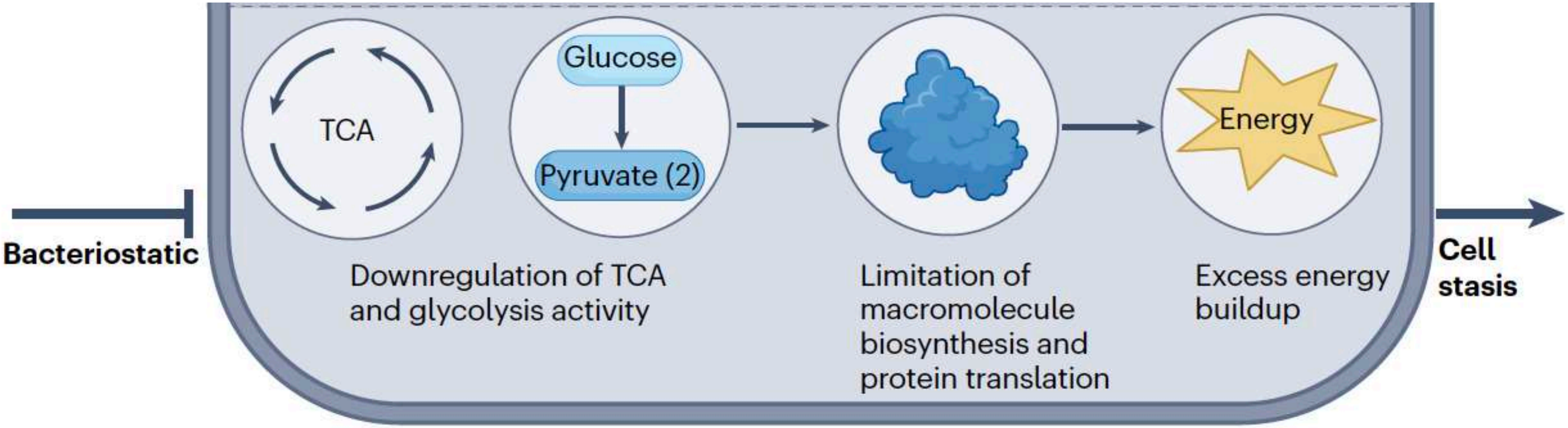


Bactericidal antibiotics induce cell death through various mechanisms that, following initial target inhibition (not shown), primarily stimulate cell metabolism, including upregulating stress response networks and electron chain and tricarboxylic acid cycle (TCA) cycle activity.

Ahmad et al., 2025

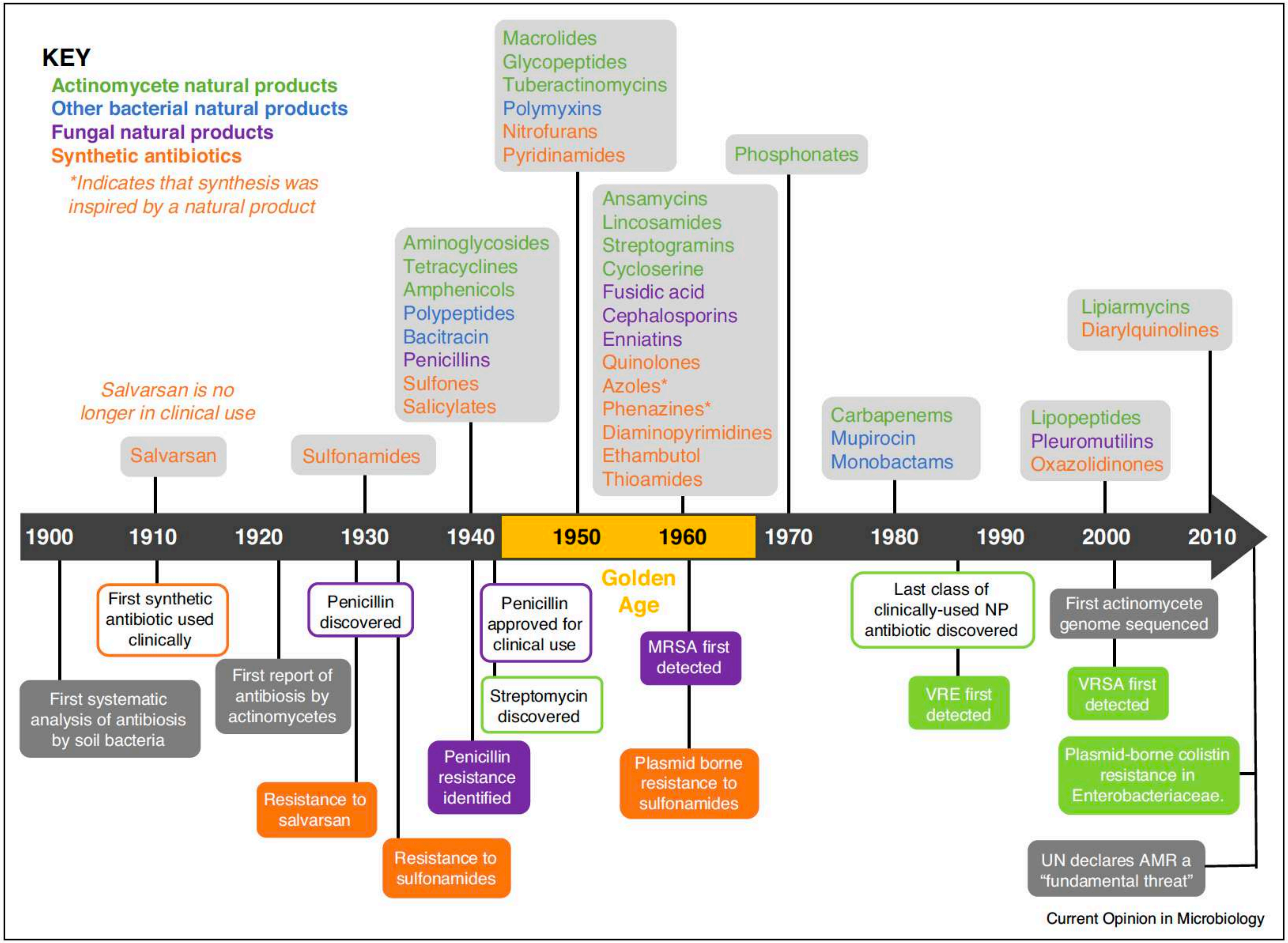
Bacteriostatic antibiotics leverage metabolism for their function

Bacteriostatic antibiotics inhibit cell growth. Classically, following initial target inhibition (not shown), growth inhibition occurs via the downregulation of key cell processes, including the glycolysis pathway and the TCA cycle. Macromolecular biosynthesis and protein translation processes may also be limited, resulting in a build-up of energy within the cell and ultimately cell stasis



Ahmad et al., 2025

Antibiotic discovery and antimicrobial resistance over time



KEY
Actinomycete natural products
Other bacterial natural products
Fungal natural products
Synthetic antibiotics
**Indicates that synthesis was inspired by a natural product*

Salvarsan is no longer in clinical use

Salvarsan

Sulfonamides

Aminoglycosides
Tetracyclines
Amphenicols
Polypeptides
Bacitracin
Penicillins
Sulfones
Salicylates

Macrolides
Glycopeptides
Tuberactinomycins
Polymyxins
Nitrofurans
Pyridinamides

Ansamycins
Lincosamides
Streptogramins
Cycloserine
Fusidic acid
Cephalosporins
Enniatins
Quinolones
Azoles*
Phenazines*
Diaminopyrimidines
Ethambutol
Thioamides

Phosphonates

Carbapenems
Mupirocin
Monobactams

Lipopeptides
Pleuromutilins
Oxazolidinones

Lipiarmycins
Diarylquinolines

1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010

Golden Age

First systematic analysis of antibiosis by soil bacteria

First synthetic antibiotic used clinically

First report of antibiosis by actinomycetes

Resistance to salvarsan

Penicillin discovered

Resistance to sulfonamides

Penicillin resistance identified

Penicillin approved for clinical use

Streptomycin discovered

MRSA first detected

Plasmid borne resistance to sulfonamides

Last class of clinically-used NP antibiotic discovered

VRE first detected

First actinomycete genome sequenced

VRSA first detected

Plasmid-borne colistin resistance in Enterobacteriaceae.

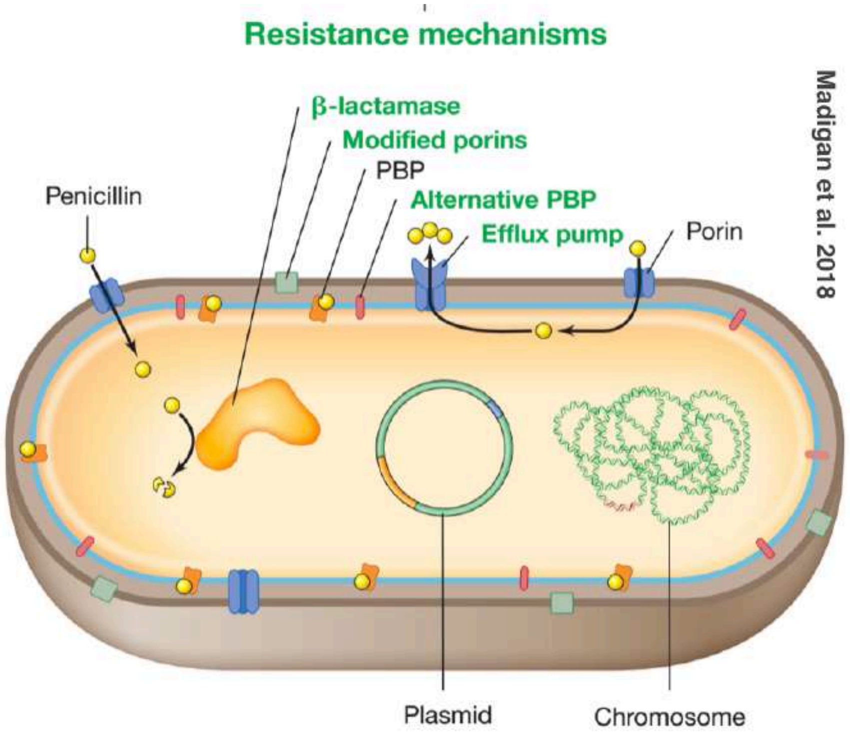
UN declares AMR a “fundamental threat”

Current Opinion in Microbiology

Hutchings et al., 2019

First reports of drug resistant strains methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae

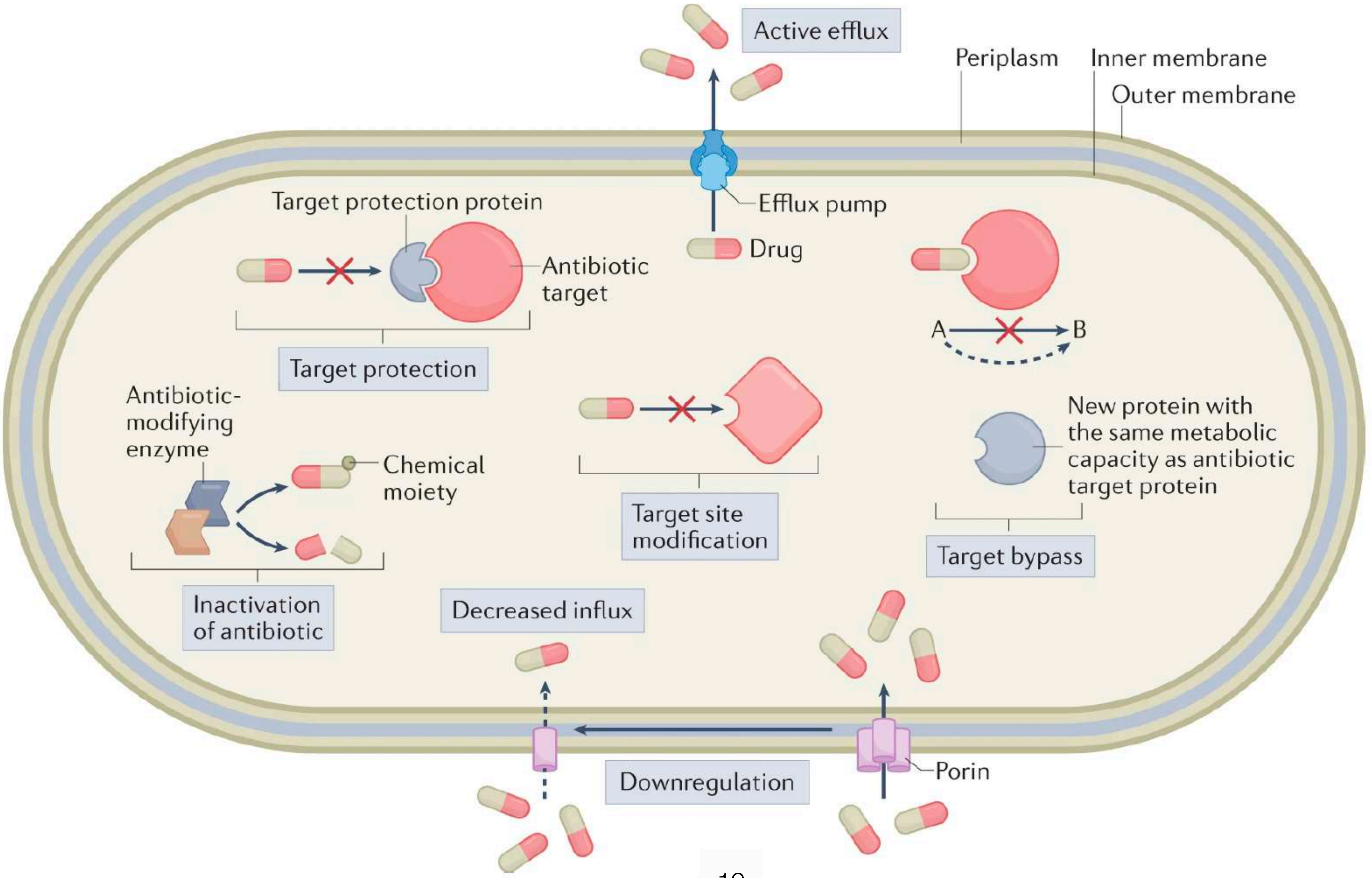
Antibiotic Resistance, I



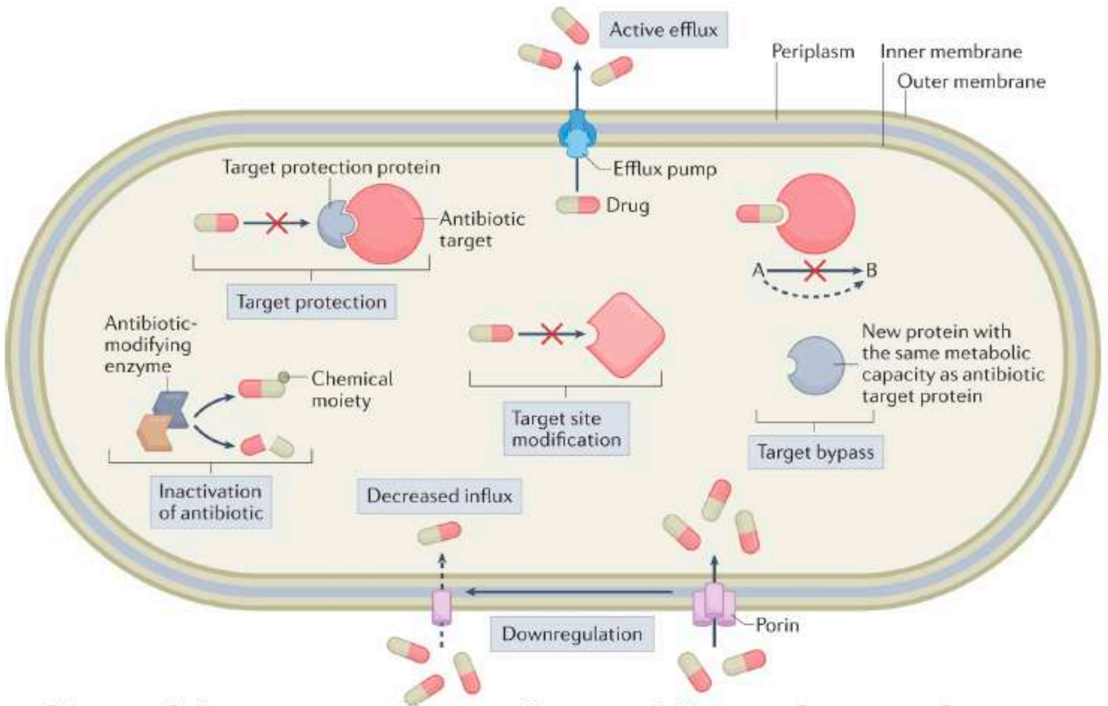
Madigan et al. 2018

- Resistance mechanisms:
    1. **Modification of the drug target**
    2. **Enzymatic inactivation**
    3. **Removal from the cell via efflux pumps**
    4. **Metabolic bypasses**
- **Random mutation** can lead to antibiotic resistance
- Resistance genes can also exist on a variety of **mobile genetic elements** and such genes can be readily transmitted between bacteria of same or different species by horizontal gene flow
- Many **mobile resistance genes, on R plasmids**, encode enzymes that **inactivate antibiotic by altering its structure, either through chemical modification or actual cleavage**: b-lactamase binds to b-lactam-type antibiotics and cleaves a key ring structure in molecule, and an acetylating enzyme adds acetyl groups to free hydroxy groups of chloramphenicol
- Pump out antibiotics that have entered the cell: efflux pumps (promiscuously)
- **Efflux** lowers intracellular concentration of an antibiotic and thus allows cell to survive at higher external concentrations: AcrAB-TolC efflux system of *E.coli* **pumps out several antibiotics** including rifampicin, chloramphenicol, fluoroquinolones

Molecular mechanisms of antibiotic resistance



Darby et al. 2022



Darby et al. 2022

Inactivation of antibiotics is mediated by enzymes that either degrade or modify the antibiotic molecule.

1. Enzymatic degradation involves hydrolysis of the functional group of the antibiotic, thereby rendering it ineffective
2. Antibiotic-modifying enzymes transfer various chemical groups to the antibiotic, which prevent binding of the antibiotic to its target
3. Target site alteration involves alteration of the antibiotic target to reduce binding of the antibiotic. This can involve mutations in the gene encoding the protein target of the antibiotic molecule or enzymatic alteration of the binding site
4. During target bypass, the function of the antibiotic target is accomplished by a new protein that is not inhibited by the antibiotic, making the original target redundant and the antibiotic ineffective
5. Decreased influx is mediated by changes to membrane structure, for example, the downregulation of porins, which are transmembrane proteins that allow the passive transport of various compounds, such as antibiotics, into the bacterial cell
6. Active efflux is facilitated by transmembrane efflux pumps, which export antibiotics out of bacterial cells to reduce their intracellular concentration
7. Target protection generally involves the physical association of a target protection protein with the antibiotic target, thereby relieving it from antibiotic-mediated inhibition

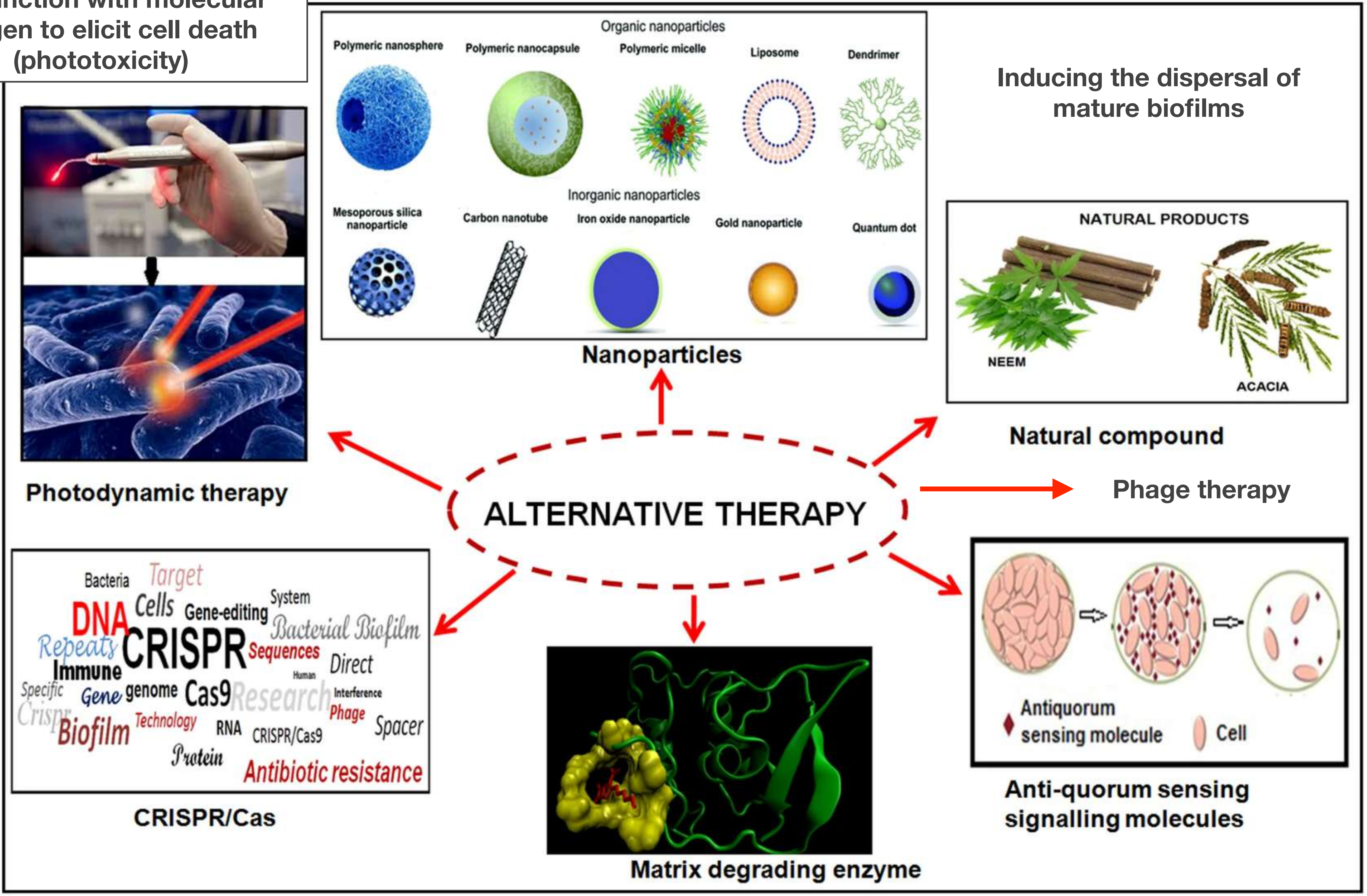
Antibiotic Resistance II

Life style: biofilm, living together in a polysaccharide matrix —> increase in antibiotic resistance (reduce permeability of antibiotics)
Life style: **shifting** to growth shut down, to **lower metabolism**
Life style: shifting to growth shut down**, stress response**

1. **Antibiotic resistance can arise both from mutations in the pre-existing genome of a bacterium and from the uptake of foreign DNA**
2. ***Antibiotic resistance is thought to have evolved long before naturally occurring antibiotics and their derivatives were used to treat human disease***
3. **ARGs (antibiotic resistance gene) have probably evolved gradually from genes with other functions**
4. ***Constant selective pressure due to antibiotic use as human therapy poses a continuous challenge that the microbes must overcome to survive***

Defeating Antibiotic Resistance

Light and a photosensitizing chemical substance, used in conjunction with molecular oxygen to elicit cell death (phototoxicity)



ALTERNATIVE THERAPY

Photodynamic therapy

Nanoparticles

Inducing the dispersal of mature biofilms

Natural compound

Phage therapy

CRISPR/Cas

Matrix degrading enzyme

Anti-quorum sensing signalling molecules

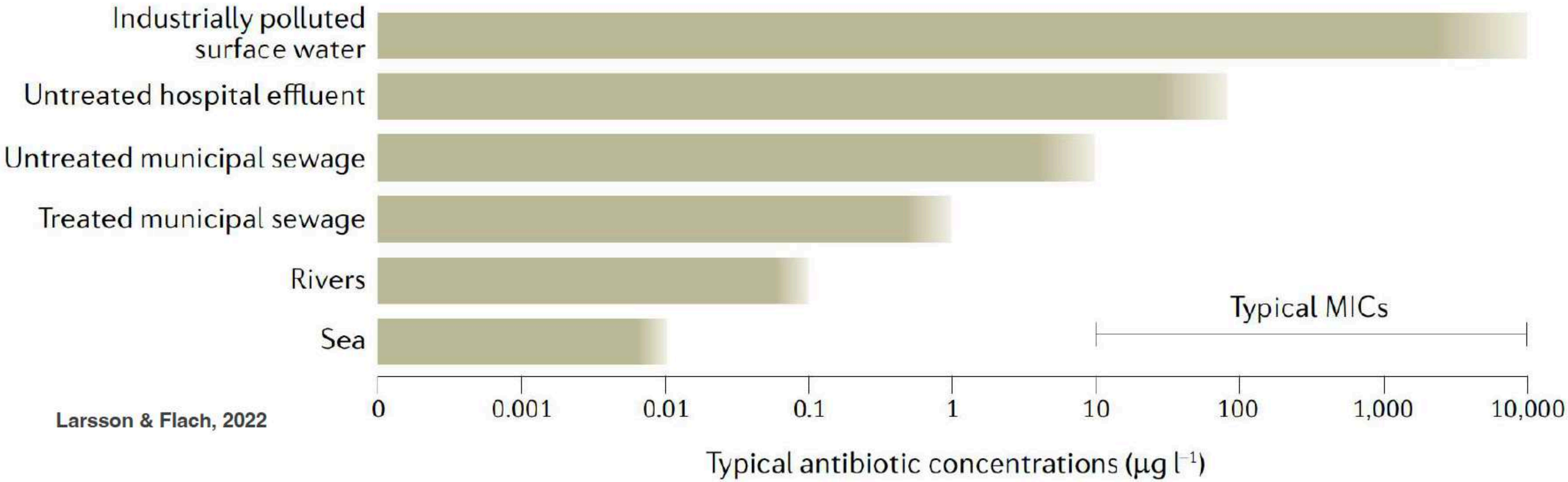
Sharma et al., 2019

AMR, AntiMicrobial Resistance
ARGs, Antimicrobial Resistance Genes
Antibiotic crisis

Antibiotic occurrence in our society

- Hospital
- At home (supervised or not supervised)
- Agriculture
- Chemical and pharmaceutical Industry
- Farming
- Wastewater treatment plants

Antibiotic concentrations in the environment

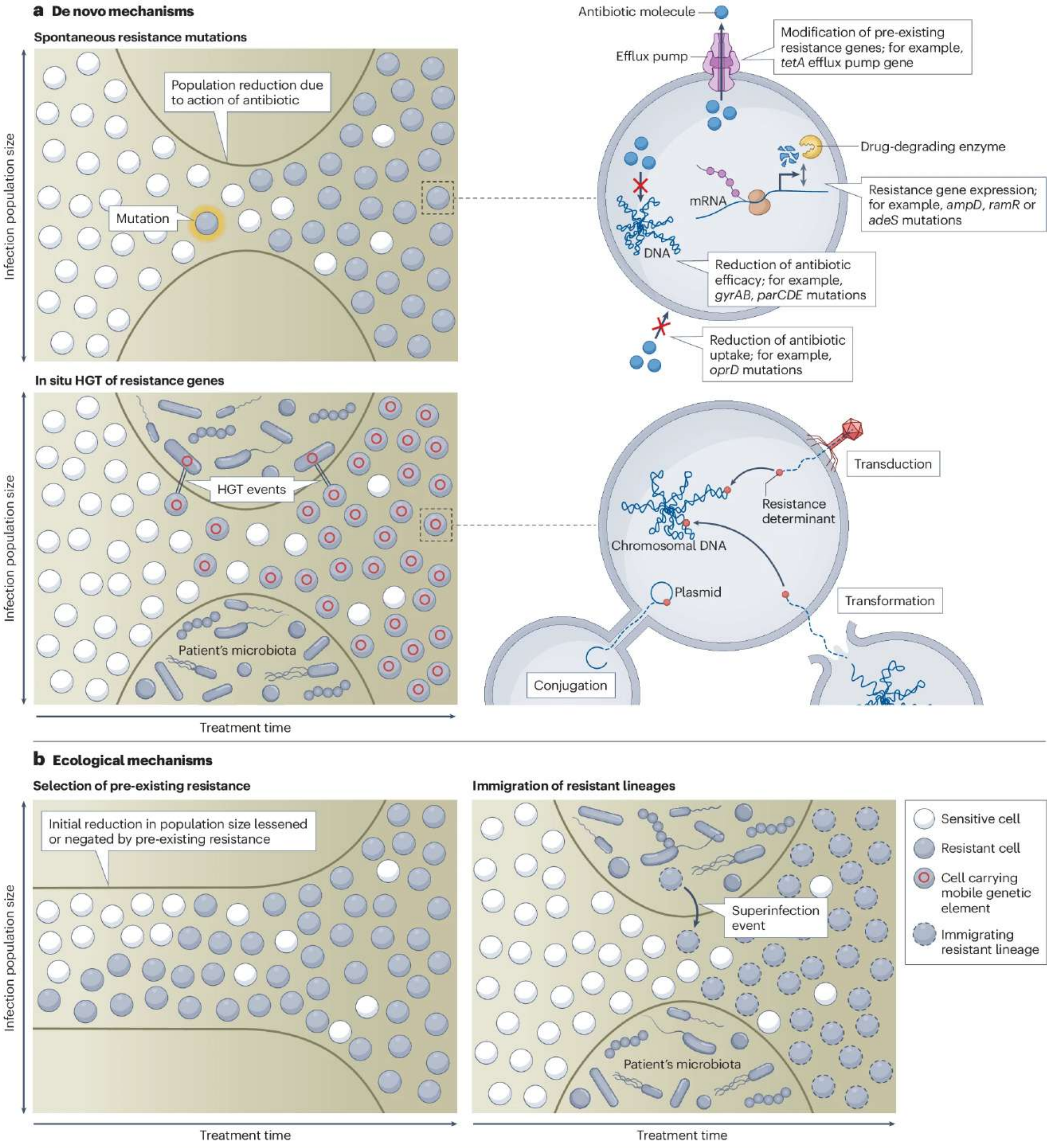


Larsson & Flach, 2022

Typical minimal inhibitory concentrations (MICs) for many antibiotic–pathogen combinations often fall within the 10–10000 µg L^{-1} range

Larssen et al., 2022

Four ecological and evolutionary mechanisms of within-patient emergence of antimicrobial resistance



Factors affecting ecological and evolutionary mechanisms

Patient characteristics
- Medical history, especially prior antimicrobial therapy
- Microbiome composition
- Action of patient's immune system
- Carriage of resistant strains or species, and MGEs

Infection characteristics
- Pathogen species and presence of competitors
- Infection duration, formation of biofilms and persisters
- Infection site 'openness' and drug penetration
- Pre-treatment presence of resistance

Shepherd et al., 2024

Resistance emergence through de novo evolutionary mechanisms: spontaneous mutation and horizontal gene transfer (HGT).

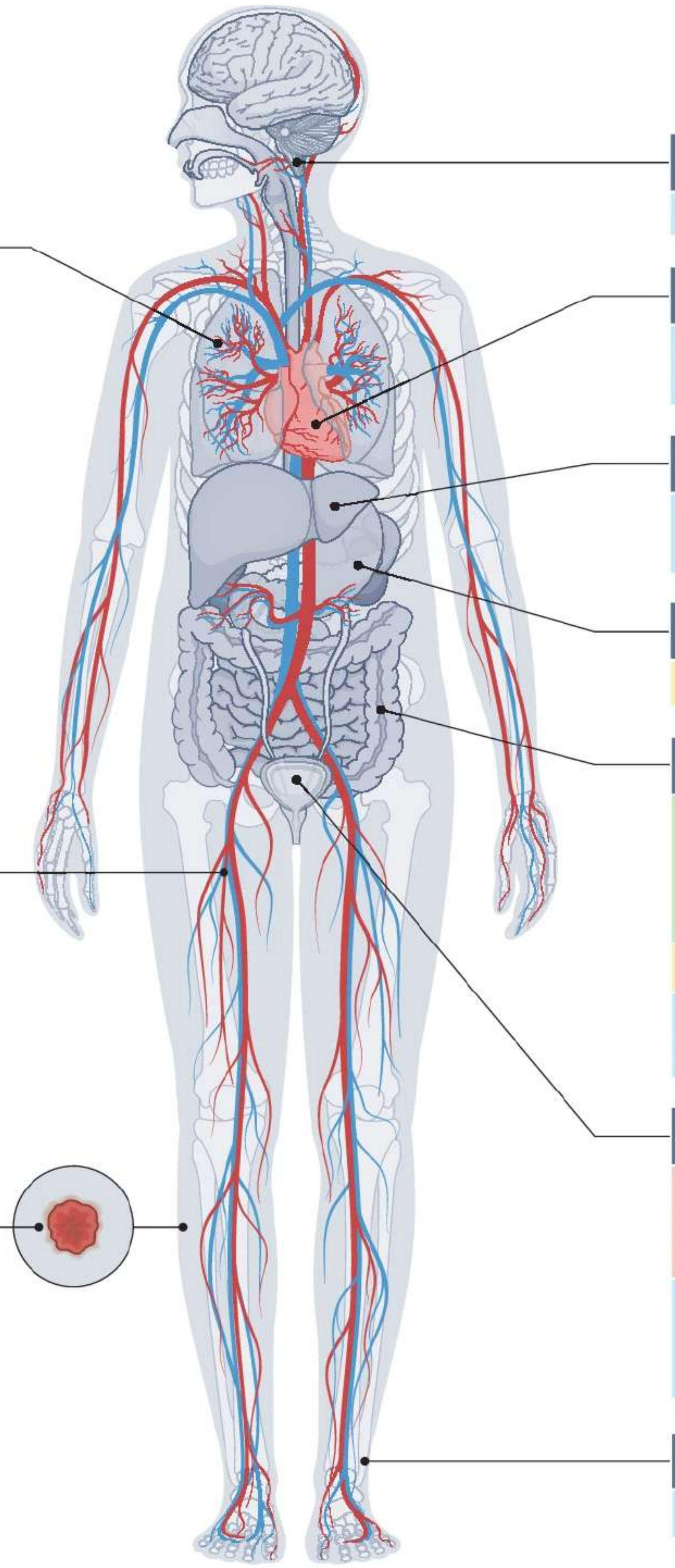
Upon commencing antimicrobial treatment, the infecting bacterial population will decline (indicated in the left-hand panels by the number of sensitive cells (white) between the black lines, with treatment time running left to right) owing to the negative effect of therapy and/or the immune system on bacterial growth. Spontaneous mutations arise continuously at random within a bacterial population, and if a mutation occurs that reduces susceptibility to the antibiotic treatment, these nascent resistant cells (grey) will then increase in frequency along with an expansion in the infection population owing to escape from the effects of therapy. These cells will have gained a resistance determinant. Common mechanistic bases of these are indicated within the highlighted cells on the right-hand side. Spontaneous mutations will typically act through reduction in antibiotic efficacy, for example, by reducing drug binding or drug uptake; regulatory mutations affecting resistance-gene expression or the activity of resistance determinants, for example, efflux pumps; and modification of pre-existing resistance genes to enhance or modify the spectrum or level of resistance they confer, for example, modification to drug-inactivating enzymes or export pumps. Spontaneous mutations often incur fitness costs that negatively affect growth of resistant cells in laboratory conditions, which may affect their survival within patients. Horizontal transfer of resistance genes within patients occurs through three key mechanisms: conjugal transfer of plasmids, bacteriophage transduction or uptake of DNA from the cell's environment (natural competence).

Shepherd et al., 2024

Ecological mechanisms of resistance emergence.

Selection of pre-existing resistance will occur immediately upon start of antibiotic treatment and may reduce the impact of treatment on bacterial population size. Resistant cells (grey) will increase in frequency as sensitive cells (white) decline in frequency as treatment progresses, and the infecting population will continue to expand, escaping the inhibitory effect of the drug. Pre-existing resistant cells may be stably present at an appreciable frequency within an infecting population owing to prior treatment with the same antibiotic, and owing to their longer evolutionary history may already have undergone compensatory evolution to reduce fitness costs of resistance. In the case of immigration of resistant lineages, a resistant strain or species will be transferred to the infection site during therapy, which may occur from the host microbiota or from elsewhere. This may occur at any time; however, as the infection is cleared by the antimicrobial treatment, this reduces competition for an invading bacterial strain or species, which may aid its establishment at the infection site. The resistant lineage is then selected for, and the infecting population of this lineage may expand. Factors that can affect the probability and action of these mechanisms of within-patient emergence of antimicrobial resistance (AMR) are listed beneath the panels, broken down into patient and pathogen. A patient's medical history (in particular prior treatment with antimicrobials) and the nature of their infection will substantially affect the likelihood of these ecological and evolutionary modes of AMR emergence. MGE, mobile genetic element.

Shepherd et al., 2024



Lung

🟦 *Pseudomonas aeruginosa* — PIP–TAZ, AZT, MER, CFD, CIP, AMP, TOB, COL, CFD–AVI
Mycobacterium tuberculosis — RIF, STR, ETH, ISO, ETN, AMI, CIP
Klebsiella pneumoniae — TIG
Haemophilus influenzae — TRI–SUL, AZI
Staphylococcus aureus — TRI–SUL, TET, LEV, RIF, LIN
Achromobacter spp. — AZT, CFT, CIP, PEN, RIF, TOB, TRI
Burkholderia pseudomallei — TRI–SUL, DOX, CFD
Stenotrophomonas maltophila — LEV, AMI, MIN, CIP, CLR

🟨 *Pseudomonas aeruginosa* — PIP–TAZ, CFD, CIP, AZT, MER, GEN
Mycobacterium tuberculosis — RIF, ISO, ETH, STR, PYR

🟥 *Pseudomonas aeruginosa* — MET
Burkholderia dolosa — CIP
Klebsiella pneumoniae — IMI, MET

Bloodstream

🟦 *Pseudomonas aeruginosa* — CFD–AVI
Klebsiella pneumoniae — PIP–TAZ, MER, DOR, FLO, MIN, CFO, CEX, TIG, PMB
Staphylococcus aureus — VAN, DAP, LIN, RIF, CIP, IMI

🟥 *Enterococcus faecium* — VAN

🟩 *Klebsiella pneumoniae* — TIG

Wounds, burns or soft tissue

🟦 *Acinetobacter baumannii* — MER

🟥 *Staphylococcus aureus* — TRI–SUL, CFT, AMO–CLV, CFX, CIP, TOB
Enterobacter hormaechei — CLN, DAP

Cerebrospinal fluid

🟦 *Staphylococcus capitis* — RIF

Heart

🟦 *Staphylococcus aureus* — RIF, DAP, VAN
Staphylococcus epidermidis — DAP

Liver and biliary system

🟦 *Escherichia coli* — CIP, CFD, IMI, TRI
Pseudomonas aeruginosa — CFD–AVI, CFZ–TAZ

Stomach

🟨 *Helicobacter pylori* — CIP, LEV, CLA, MET

Gut

🟩 *Clostridium difficile* — CLN
Klebsiella pneumoniae — MIN, LEV, IMI
Escherichia coli — AMO
Salmonella enterica — CFT

🟨 *Enterococcus* spp. — TOB, COL

🟦 *Pseudomonas aeruginosa* — MER
Campylobacter jejuni — AMO–CLV, CIP

Urinary tract

🟥 *Escherichia coli* — TRI–SUL, CIP, AMO–CLV, CEP, CEF
Klebsiella pneumoniae — FOS, NIT
Proteus mirabilis — NIT

🟦 *Escherichia coli* — NIT, CFT, CFX, IMI, CIP, AMO–CLV, TRI–SUL, FOS
Klebsiella pneumoniae — TIG

Bone

🟦 *Cedecea davisae* — AMO–CLV

🟦 Spontaneous mutation	🟩 Horizontal gene transfer
🟨 Selection of pre-existing resistance	🟥 Immigration of resistant lineages

Shepherd et al., 2024

Studies of within-patient antimicrobial resistance (AMR) emergence included in this Review cover a wide range of body sites, antimicrobials and bacterial pathogens.

Reports of spontaneous resistance evolution are the largest single group, and the lungs are a site where resistance emergence is most frequently documented.

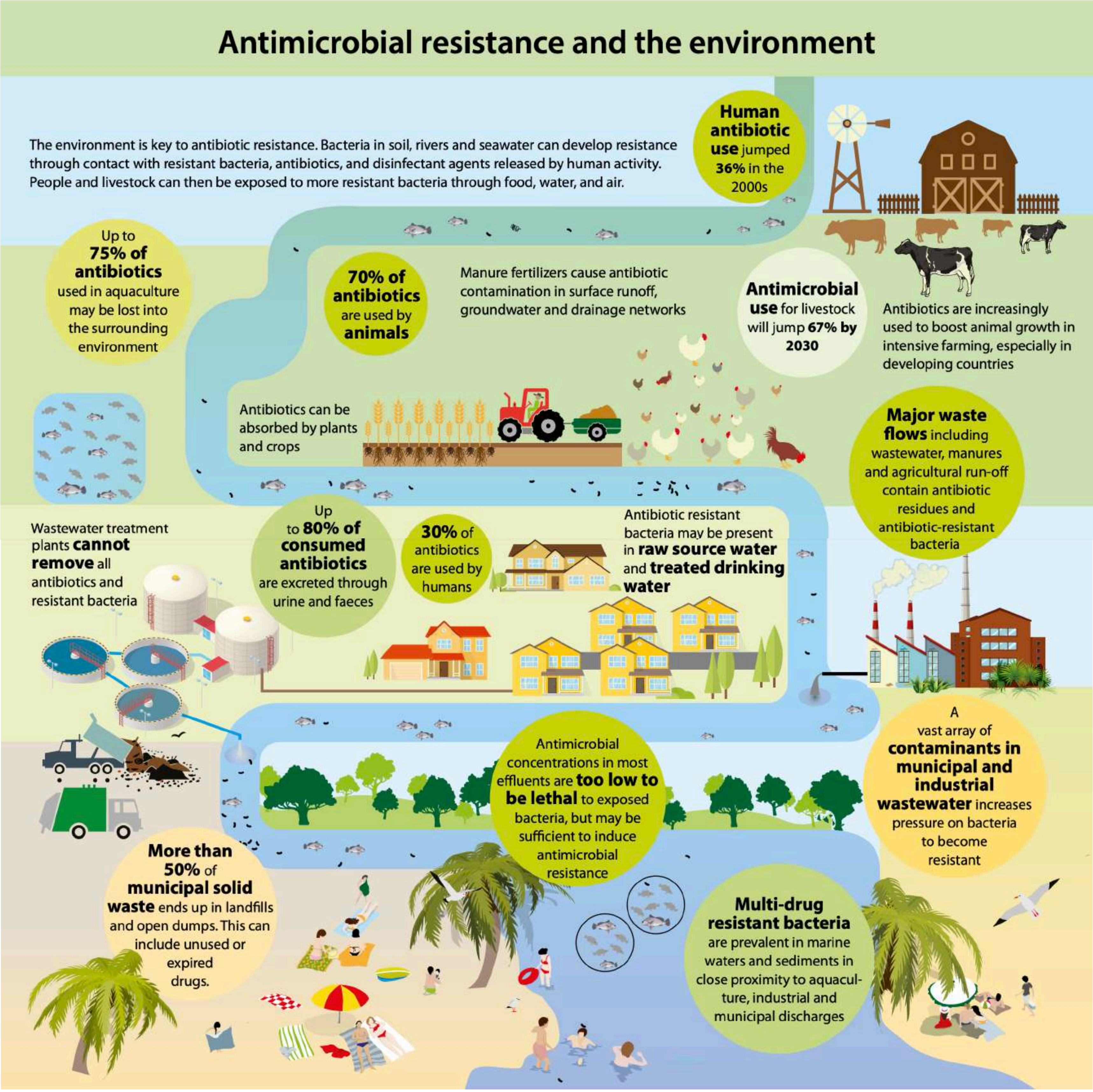
Reports for some host niches are dominated by particular ecological and evolutionary mechanisms, for example, horizontal gene transfer in the gut and spontaneous mutation in more isolated infection sites including bone, cerebrospinal fluid, heart and liver.

However, it should be noted that the distribution of ecological and evolutionary modes, sites and pathogens may reflect biases due to ease of study, methodologies used or other factors.

AMI, amikacin; AMO, amoxicillin; AMO–CLV, amoxicillin–clavulanate; AMP, ampicillin; AZI, azithromycin; AZT, aztreonam; CEF, cefuroxime axetil; CEX, cefotaxime; CEP, cephalexin; CFD, ceftazidime; CFD–AVI, ceftazidime–avibactam; CFO, cefazolin; CFT, ceftriaxone; CFX, cefixime; CFZ–TAZ, ceftolozane–tazobactam; CIP, ciprofloxacin; CLA, clarithromycin; CLN, clindamycin; CLR, chloramphenicol; COL, colistin; DOR, doripenem; DOX, doxycycline; DAP, daptomycin; ETH, ethambutol; ETN, ethionamide; FLO, flomoxef; FOS, fosfomycin; GEN, gentamicin; IMI, imipenem; ISO, isoniazid; LEV, levofloxacin; LIN, linezolid; MER, meropenem; MET, metronidazole; MIN, minocycline; NIT, nitrofurantoin; PEN, penicillin; PIP–TAZ, piperacillin–tazobactam; PMB, polymyxin B; PYR, pyrazinamide; RIF, rifampicin; STR, streptomycin; TET, tetracycline; TIG, tigecycline; TOB, tobramycin; TRI, trimethoprim; TRI–SUL, trimethoprim–sulfamethoxazole; VAN, vancomycin.

Shepherd et al., 2024

Antimicrobial resistance global crisis



Antimicrobial resistance and the environment

The environment is key to antibiotic resistance. Bacteria in soil, rivers and seawater can develop resistance through contact with resistant bacteria, antibiotics, and disinfectant agents released by human activity. People and livestock can then be exposed to more resistant bacteria through food, water, and air.

Human antibiotic use jumped **36%** in the 2000s

Up to **75% of antibiotics** used in aquaculture may be lost into the surrounding environment

70% of antibiotics are used by **animals**

Manure fertilizers cause antibiotic contamination in surface runoff, groundwater and drainage networks

Antimicrobial use for livestock will jump **67% by 2030**

Antibiotics are increasingly used to boost animal growth in intensive farming, especially in developing countries

Antibiotics can be absorbed by plants and crops

Major waste flows including wastewater, manures and agricultural run-off contain antibiotic residues and antibiotic-resistant bacteria

Wastewater treatment plants **cannot remove** all antibiotics and resistant bacteria

Up to **80% of consumed antibiotics** are excreted through urine and faeces

30% of antibiotics are used by humans

Antibiotic resistant bacteria may be present in **raw source water** and **treated drinking water**

A vast array of **contaminants in municipal and industrial wastewater** increases pressure on bacteria to become resistant

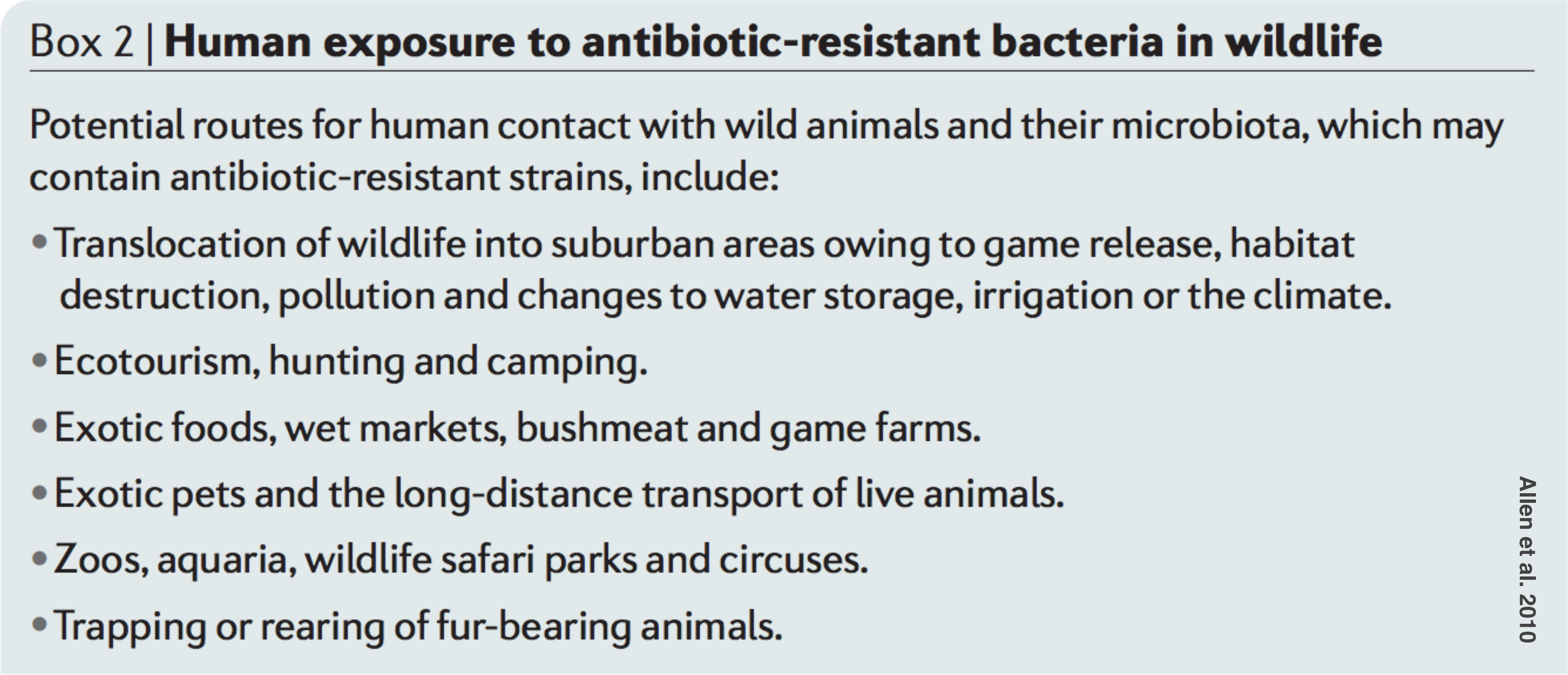
Antimicrobial concentrations in most effluents are **too low to be lethal** to exposed bacteria, but may be sufficient to induce antimicrobial resistance

More than 50% of **municipal solid waste** ends up in landfills and open dumps. This can include unused or expired drugs.

Multi-drug resistant bacteria are prevalent in marine waters and sediments in close proximity to aquaculture, industrial and municipal discharges

UN environment programme

https://www.unenvironment.org/resources/frontiers-2017-emerging-issues-environmental-concern

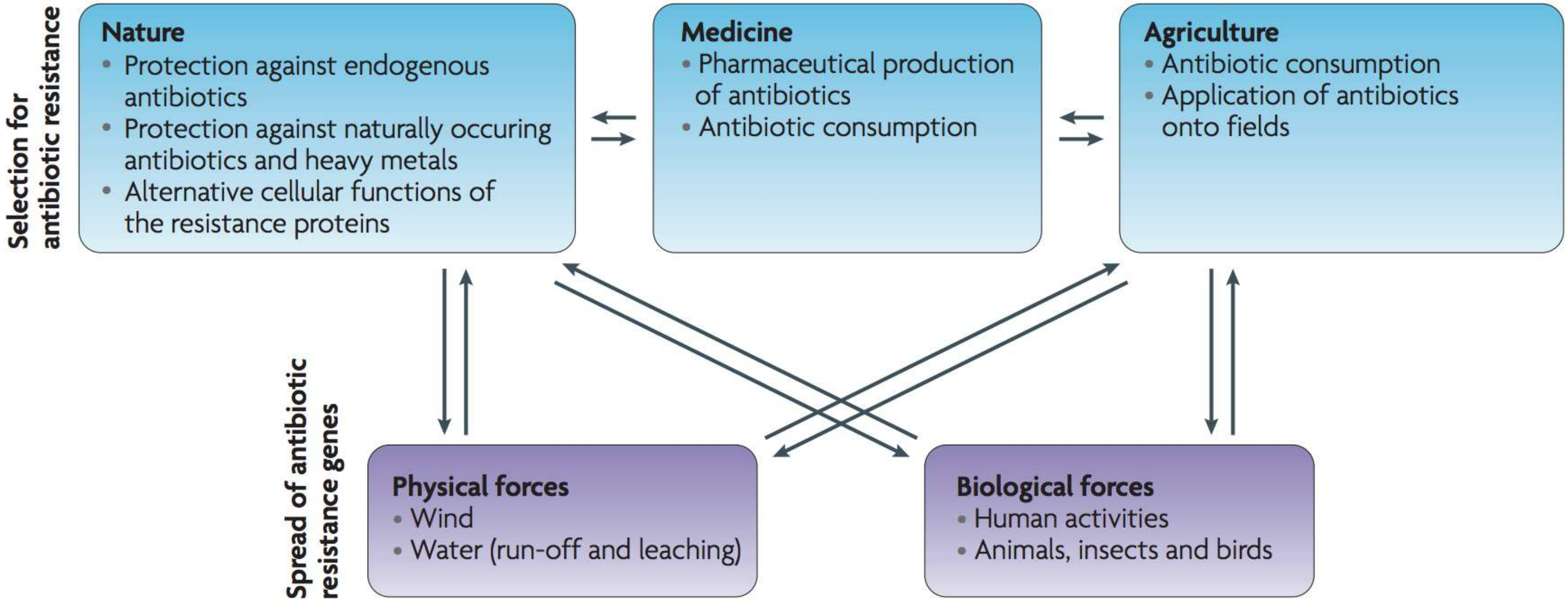


Box 2 | **Human exposure to antibiotic-resistant bacteria in wildlife**

Potential routes for human contact with wild animals and their microbiota, which may contain antibiotic-resistant strains, include:

- Translocation of wildlife into suburban areas owing to game release, habitat destruction, pollution and changes to water storage, irrigation or the climate.
- Ecotourism, hunting and camping.
- Exotic foods, wet markets, bushmeat and game farms.
- Exotic pets and the long-distance transport of live animals.
- Zoos, aquaria, wildlife safari parks and circuses.
- Trapping or rearing of fur-bearing animals.

Allen et al. 2010



Selection for antibiotic resistance

Nature
- Protection against endogenous antibiotics
- Protection against naturally occuring antibiotics and heavy metals
- Alternative cellular functions of the resistance proteins

Medicine
- Pharmaceutical production of antibiotics
- Antibiotic consumption

Agriculture
- Antibiotic consumption
- Application of antibiotics onto fields

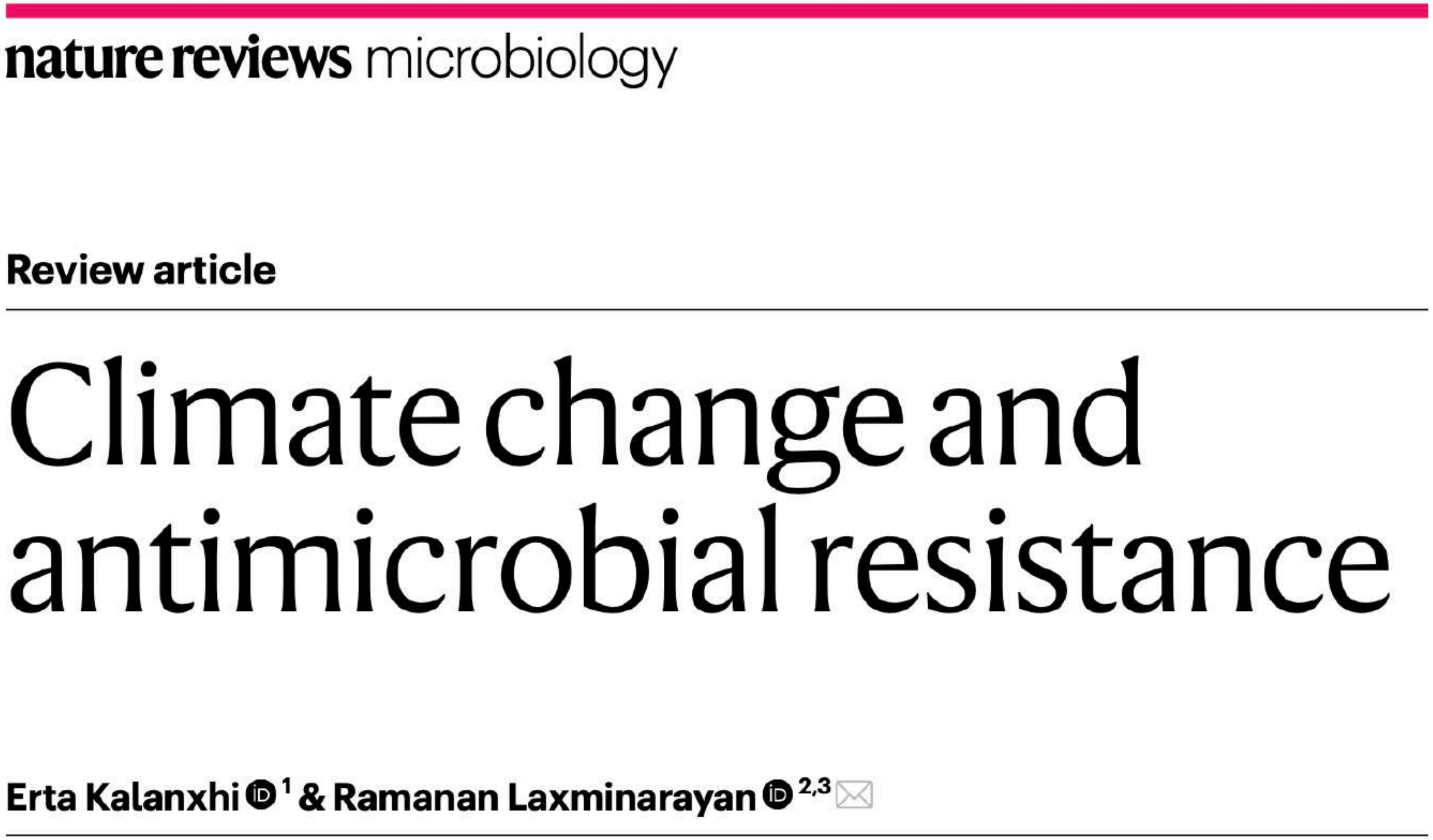
Spread of antibiotic resistance genes

Physical forces
- Wind
- Water (run-off and leaching)

Biological forces
- Human activities
- Animals, insects and birds

Figure 2 | **Sources and movement of antibiotic resistance genes in the environment.** Resistance genes exist naturally in the environment owing to a range of selective pressures in nature. Humans have applied additional selective pressure for antibiotic resistance genes because of the large quantities of antibiotics that we produce, consume and apply in medicine and agriculture. Physical and biological forces also cause widespread dissemination of resistance genes throughout many environments.

Allen et al. 2010



nature reviews microbiology

Review article

Climate change and antimicrobial resistance

Erta Kalanxhi[1] & Ramanan Laxminarayan[2,3] ✉

2026

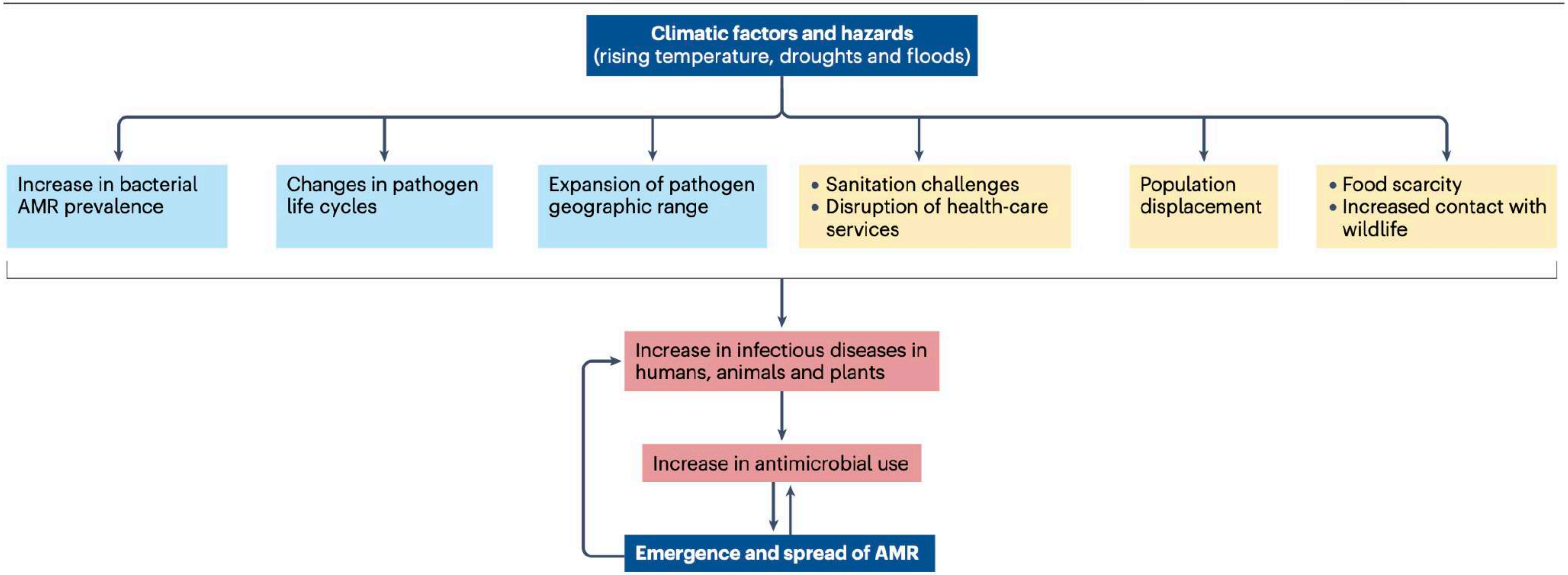
- The decreasing effectiveness of antibiotics owing to antimicrobial resistance (AMR) threatens the progress made in reducing infectious disease mortality since the drugs were first used in the 1940s

- About two-thirds of approximately 7.7 million deaths from bacterial infections each year are associated with AMR, with roughly one-sixth directly attributable to drug-resistant infections

- Invasive fungal infections affect 6.5 million people annually and are associated with 3.8 million deaths, many of which are owing to antifungal resistance

- AMR is a global challenge, its burden varies greatly across countries, partly owing to disparities in access to water, sanitation and hygiene (WASH) infrastructure, diagnostics, vaccines and antimicrobials

Warming climates and extreme weather events exacerbate infectious disease burden and antimicrobial use



Climatic factors and hazards
(rising temperature, droughts and floods)

Increase in bacterial AMR prevalence

Changes in pathogen life cycles

Expansion of pathogen geographic range

- Sanitation challenges
- Disruption of health-care services

Population displacement

- Food scarcity
- Increased contact with wildlife

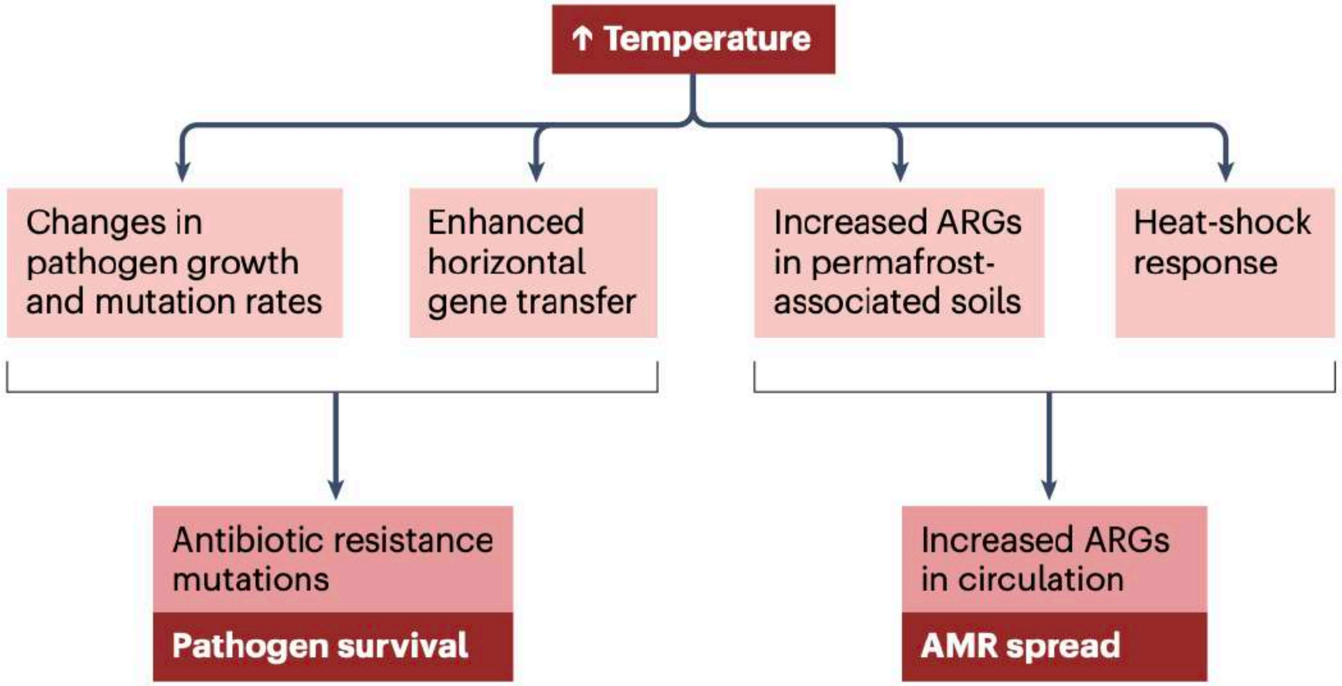
Increase in infectious diseases in humans, animals and plants

Increase in antimicrobial use

Emergence and spread of AMR

Kalanxhi and Laxminarayan, 2026

Direct effects of temperature on the emergence and spread of antimicrobial resistance



↑ Temperature

- Changes in pathogen growth and mutation rates
- Enhanced horizontal gene transfer
- Increased ARGs in permafrost-associated soils
- Heat-shock response

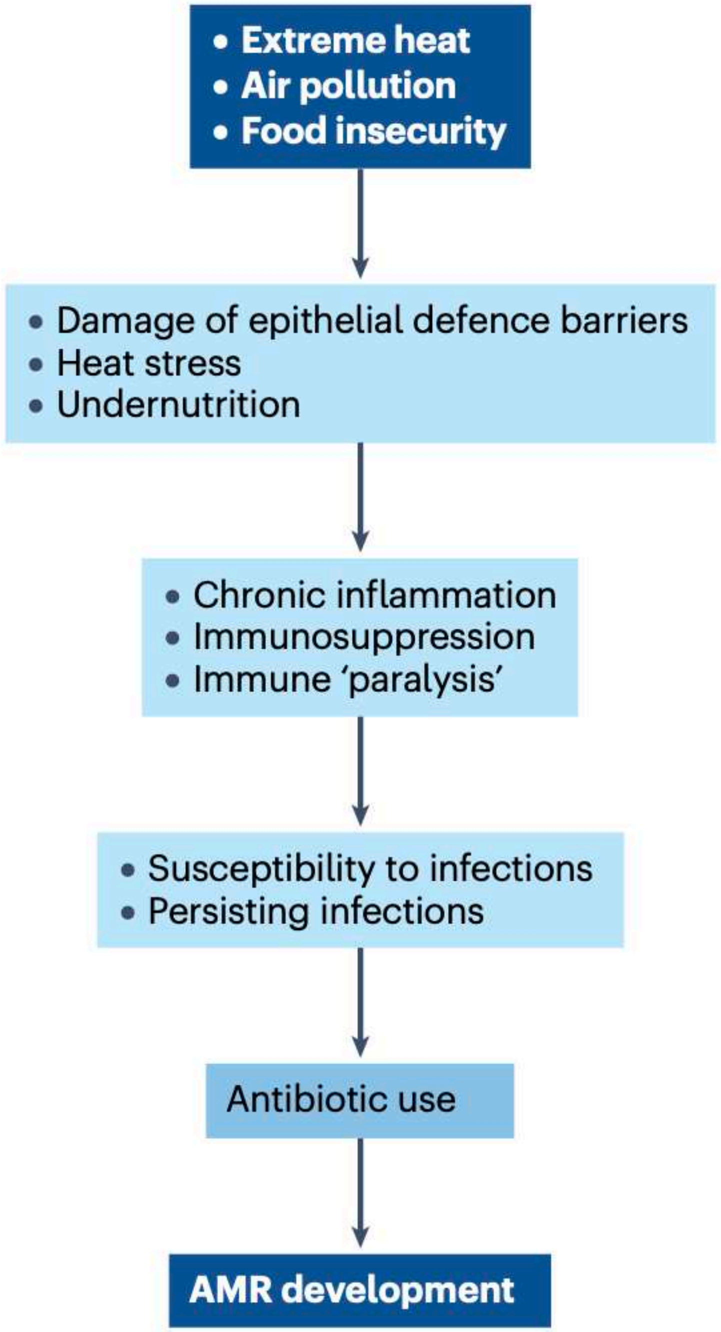
Antibiotic resistance mutations

Pathogen survival

Increased ARGs in circulation

AMR spread

Effects of climate change on host immunity



- **Extreme heat**
- **Air pollution**
- **Food insecurity**

- Damage of epithelial defence barriers
- Heat stress
- Undernutrition

- Chronic inflammation
- Immunosuppression
- Immune ‘paralysis’

- Susceptibility to infections
- Persisting infections

Antibiotic use

AMR development

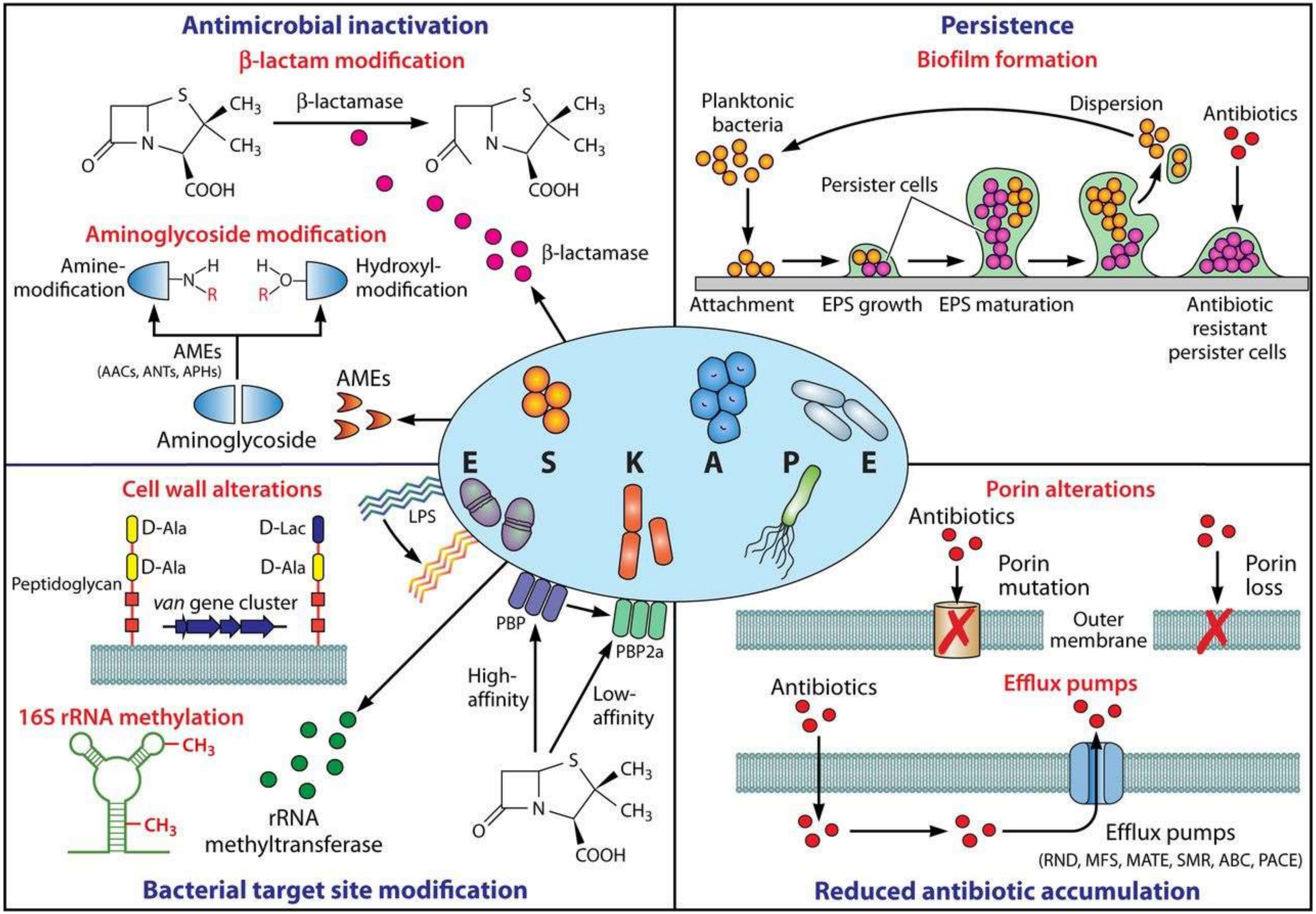
Kalanxhi and Laxminarayan, 2026

Super bugs

Megaplate experiments

Mediators of ESKAPE pathogen antimicrobial resistance

ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species)

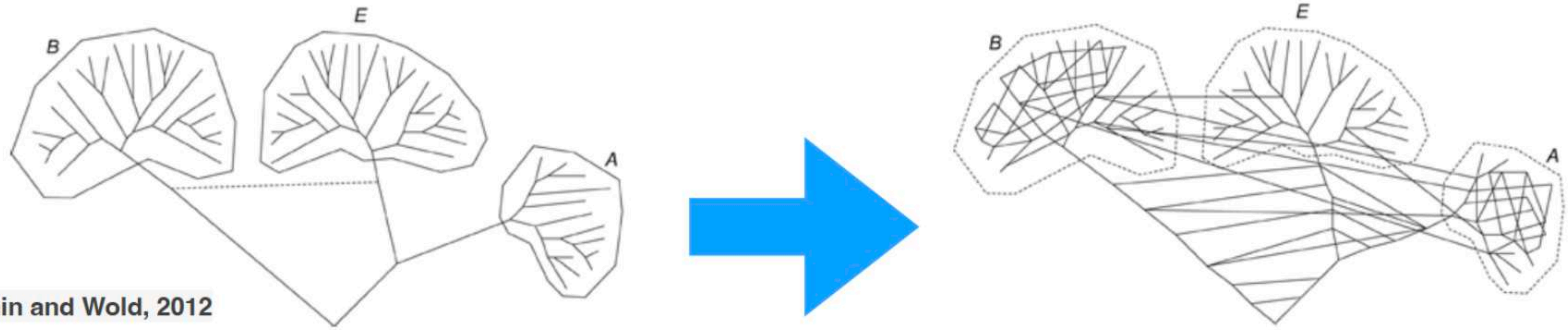


David M. P. De Oliveira et al. Clin. Microbiol. Rev. 2020; doi:10.1128/CMR.00181-19

To be or not to be?

Theodosius Dobzhansky: “Nothing in biology makes sense except in the light of evolution”

Paradigm shift:
Vertical gene transfer
Horizontal gene transfer



Koonin and Wold, 2012

Genetic Elements

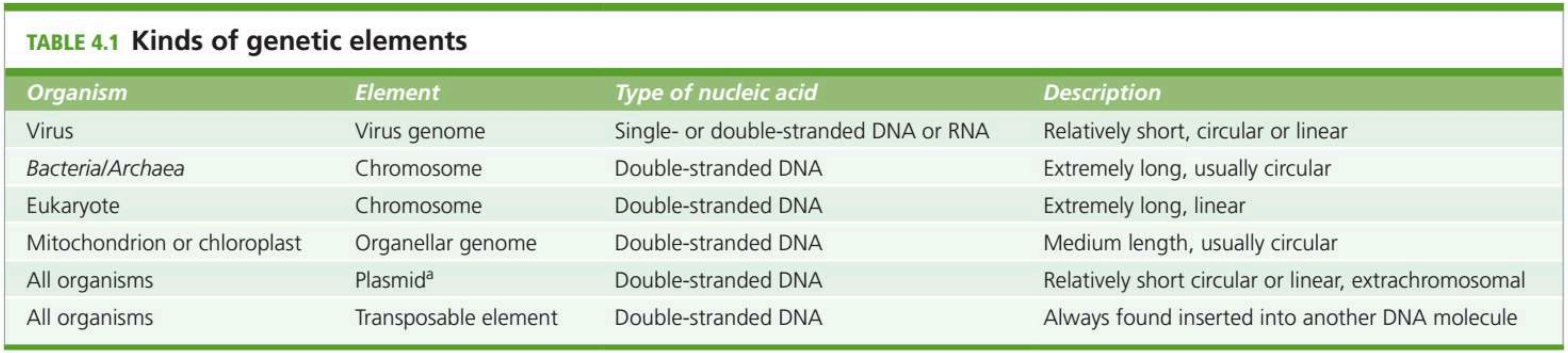
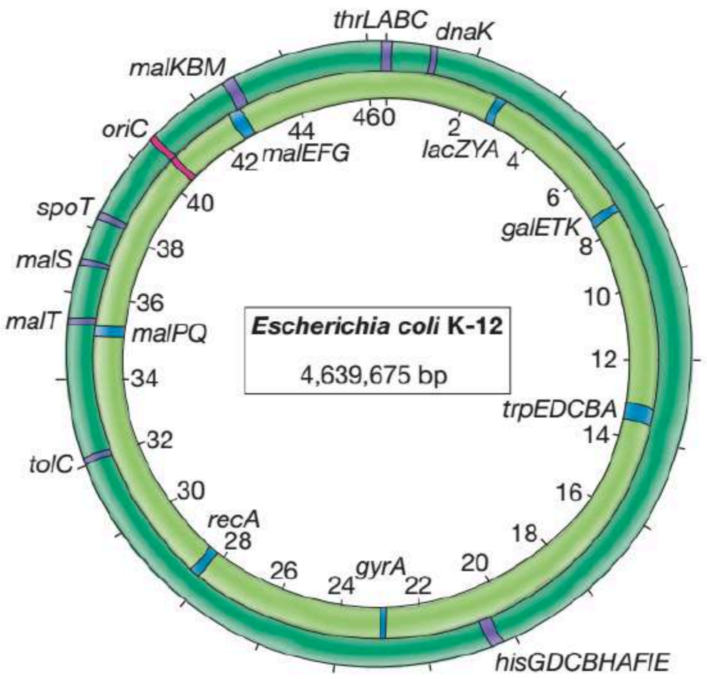


TABLE 4.1 Kinds of genetic elements

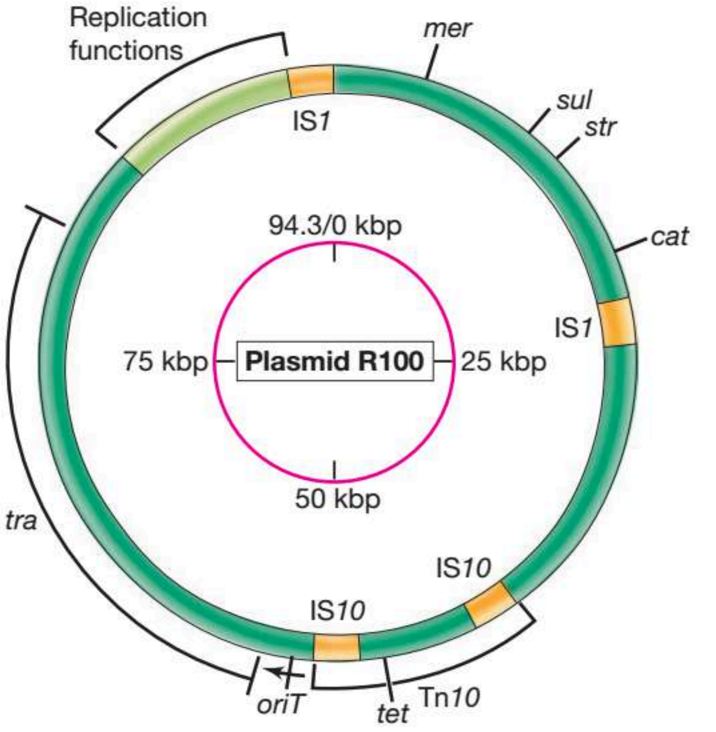
Organism	*Element*	*Type of nucleic acid*	*Description*
Virus	Virus genome	Single- or double-stranded DNA or RNA	Relatively short, circular or linear
Bacteria/Archaea	Chromosome	Double-stranded DNA	Extremely long, usually circular
Eukaryote	Chromosome	Double-stranded DNA	Extremely long, linear
Mitochondrion or chloroplast	Organellar genome	Double-stranded DNA	Medium length, usually circular
All organisms	Plasmid[a]	Double-stranded DNA	Relatively short circular or linear, extrachromosomal
All organisms	Transposable element	Double-stranded DNA	Always found inserted into another DNA molecule

[a] Plasmids are uncommon in eukaryotes.

Madigan et al. 2020



***E. coli* strain K-12**



Resistance plasmid R100

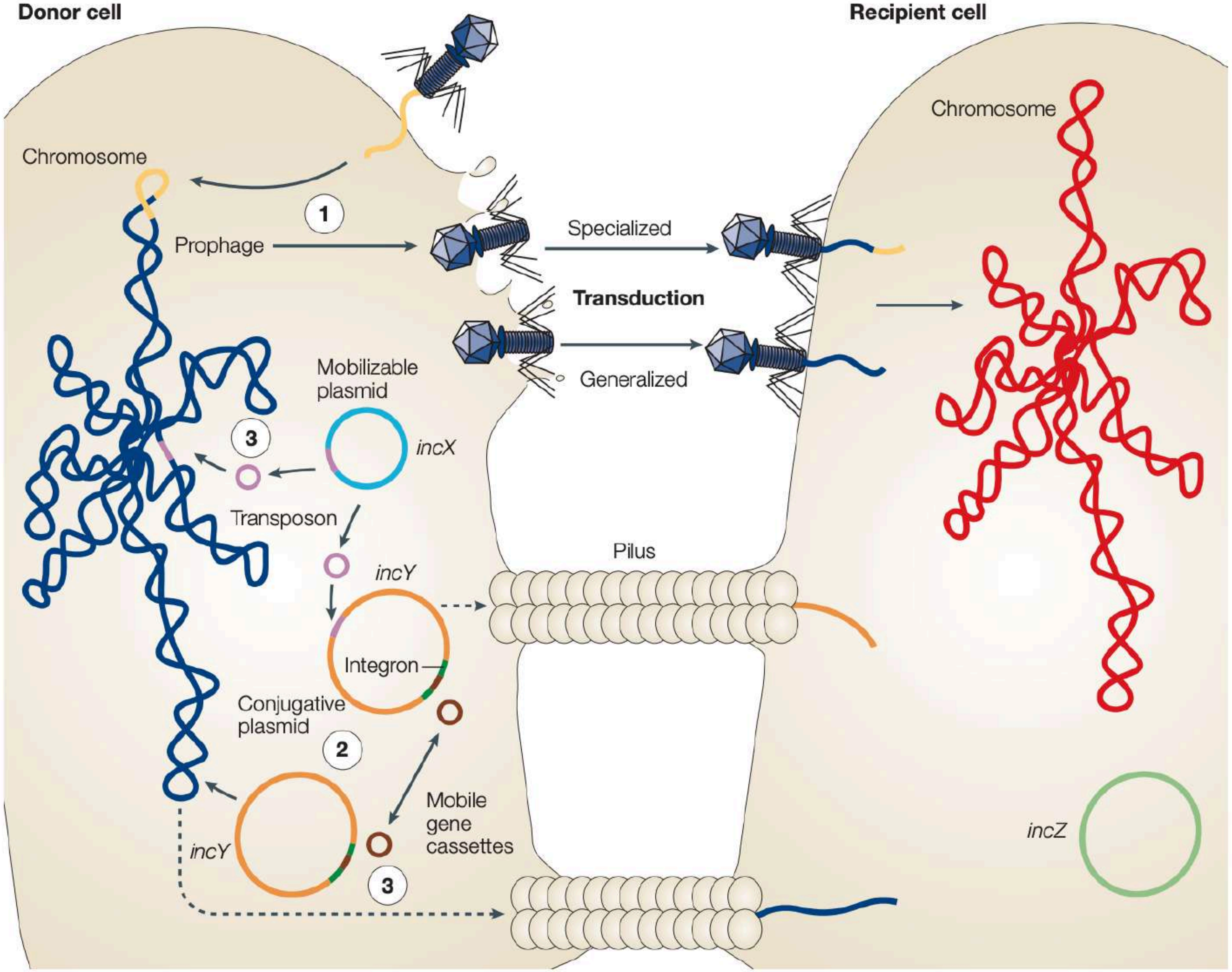
Madigan et al. 2020

Mobile Genetic Elements, I

The *mobilome*, **defined as all mobile genetic elements (MGEs) of the microbiome**, *influences the composition of microbial communities* and the *spread of antimicrobial resistance genes and virulence factors, CRISPR-Cas, Quorum sensing, Restriction-Modification, Toxin, via horizontal gene transfer (HGT) and* **contribute to evolution (by shuffling genes)**

Mobile Genetic Elements, II

Frost et al., 2005



- Integrons are genetic elements that can capture **gene cassettes (mobile DNA containing a recombination site**) through the activity of an enzyme called **integrase**
- **Transposons are mobile genetic elements** that move between different host DNA molecules, including chromosomes, plasmids, and viruses by the activity of an enzyme called **transposase**
- Transposons may pick up and horizontally transfer genes encoding various characteristics, including **resistance to antibiotics and production of toxins**
- Transposon **strong driver of genome evolution** mediate a variety of large-scale chromosomal changes

Mobile Genetic Elements, III

- **Plasmids**
- **Bacteriophages**
- **Insertion sequences (IS) and transposons (Tn)**
- **GEnomic (PAthogenicity islands, PAIs) island (GEI), Phage-inducible chromosomal island, Phage-plasmids, Gene transfer agents, Integrative and conjugative elements, integrative and mobilisable elements, Integrons**

Mobile Genetic Elements: *Plasmids*

- Most Bacteria and Archaea contain a single circular **chromosome** containing all (or most) of the organism's genes (Euk, linear DNA) and also a second small chromosome
- **Plasmids** are circular or linear double-stranded DNA molecules **that replicate separately from chromosome** and are typically much smaller than chromosomes (1 kbp to more than 1 Mbp), 5% of total genomes, present in one or more copies
    A. **Thousands of different plasmids** are known, and >300 different plasmids from strains of *E. coli*

    B. **Enzymes that replicate chromosomal DNA also replicate plasmids**. Some of genes encoded on a plasmid function to direct initiation of plasmid replication and to partition replicated plasmids between daughter cells

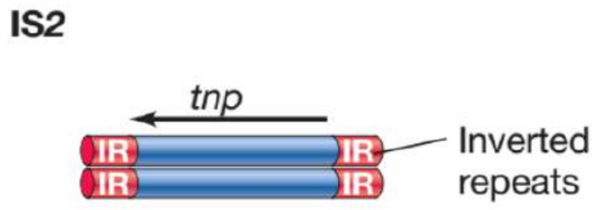
    C. **Virulent genes, antibiotic and metal resistance**, other special metabolism

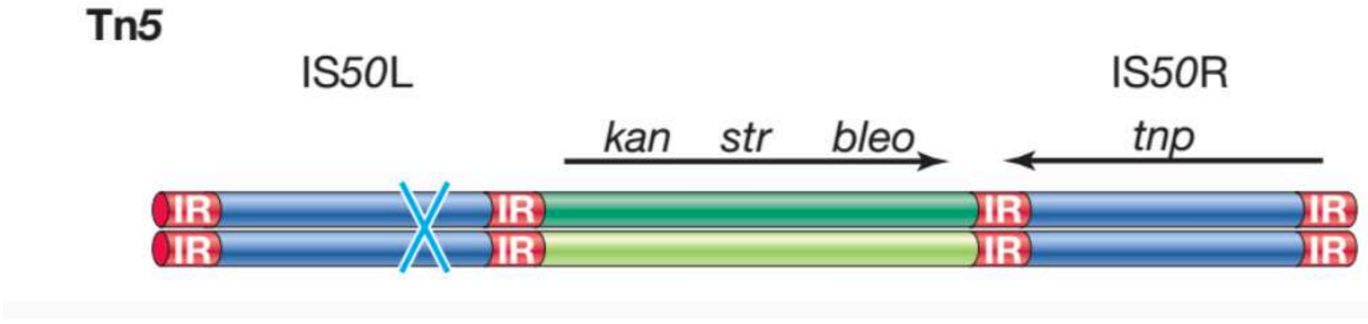
Mobile Genetic Elements: *Transposable elements*

- **Transposable elements** are sequences of DNA that are inserted into other DNA molecules but can move from one site on DNA molecule to another, either within same molecule or on a different DNA molecule. **NOT SELF-TRANSMISSABLE**
- Chromosomes, plasmids, virus genomes, and any other type of DNA molecule may host a transposable element (**Transposable element a genetic element able to move (transpose) from one site to another on host DNA molecules)**

★ **Insertion sequences** are the simplest type of transposable element: short DNA segments, ~ **1000 nucleotides long**, and typically contain inverted repeats of 10–50 base pairs, **only protein is transposase**

★**Transposon a type of transposable element that carries genes in addition to those required for transposition**) Transposons >> IS elements



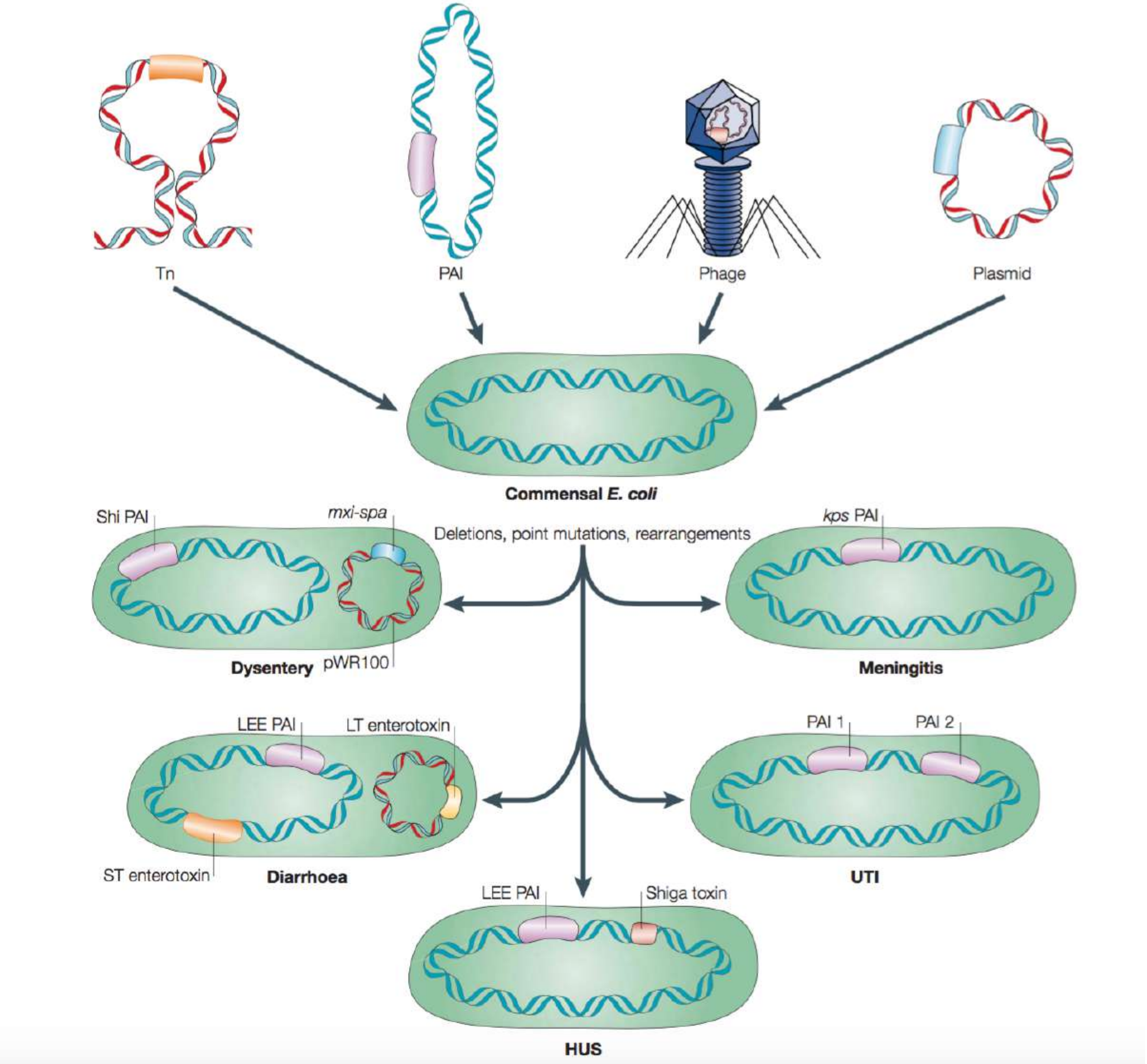


Madigan et al. 2018

Mobile Genetic Elements, IV

- **Transposable elements** are sequences of DNA that are inserted into other DNA molecules but can move from one site on DNA molecule to another, either within same molecule or on a different DNA molecule
- Chromosomes, plasmids, virus genomes, and any other type of DNA molecule may host a transposable element (**Transposable element a genetic element able to move (transpose) from one site to another on host DNA molecules; Transposon a type of transposable element that carries genes in addition to those required for transposition**)
- Bacteria undergoing **rapid evolutionary change** often contain relatively **large numbers of mobile elements**, especially insertion sequences, simple transposable elements whose genes encode only transposition
- **Recombination among identical elements** generates chromosomal rearrangements such as **deletions, inversions, or translocations** —> genomic diversity upon which natural selection can act
- **Chromosomal rearrangements** that accumulate in bacteria during **stressful growth conditions** are often flanked by repeats or insertion sequences

MGEs contribution to the evolution of pathogenic *E. coli*



MGEs additions, deletions and other genetic changes can give rise to pathogenic *E. coli* forms capable of causing:

1 diarrhoea (EPEC, EHEC, EAEC DAEC),

2 dysentery (EIEC)

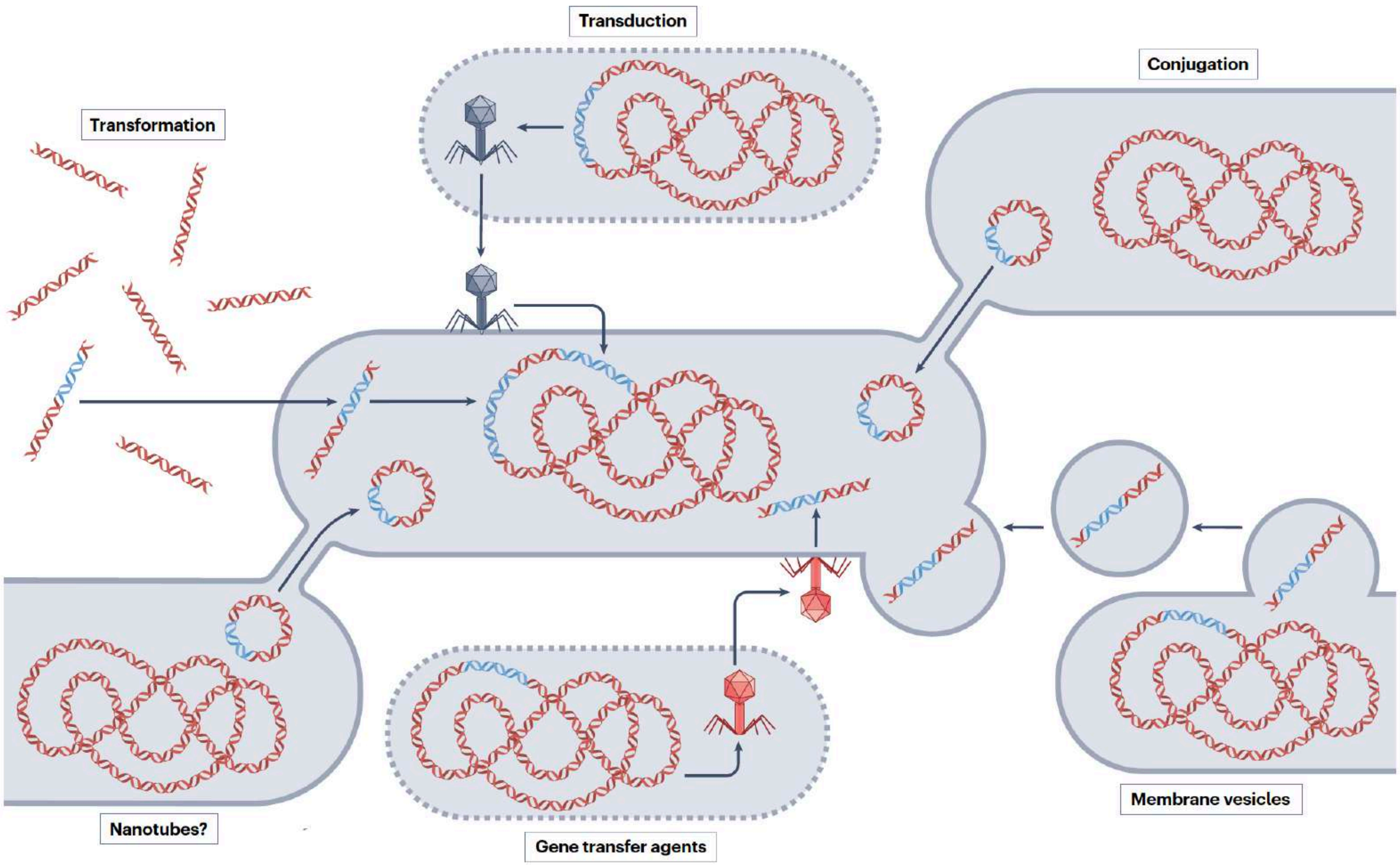
3 haemolytic uremic syndrome, hus (EHEC),

3 urinary tract infections, uti (UPEC)

4 meningitis (MNEC)

Kasper et al., 2004

Mechanisms of horizontal gene transfer

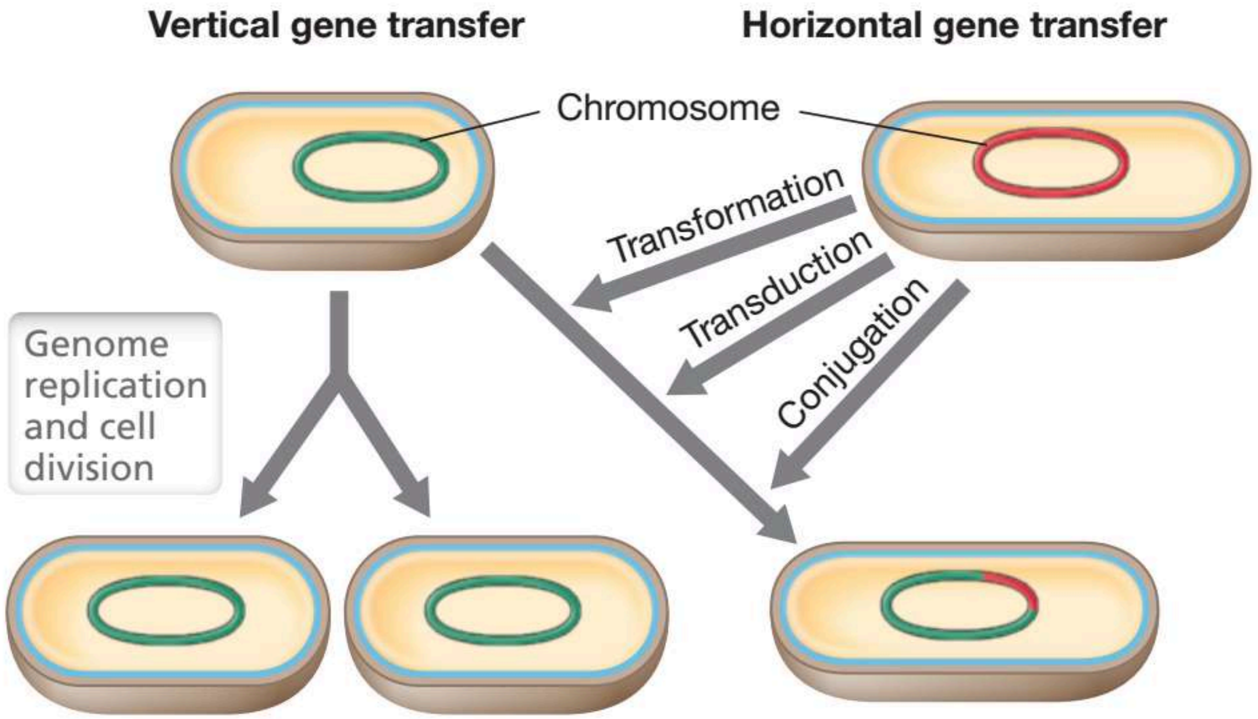


Castañeda-Barba et al., 2024

Transformation is the uptake of exogenous DNA from the environment; *transduction is the virus-mediated transfer of genetic material*; **conjugation is plasmid or conjugative element-mediated transfer through cell–cell contact;** *nanotubes are membranous structures that bridge bacteria and enable exchange of genetic material*; **gene transfer agents are virus-like particles that can mediate gene transfer**; and **membrane vesicles are rounded nanostructures secreted by Gram-negative bacteria** that can behave as molecular carriers for genes

Horizontal Gene Transfer (HGT), I

- **Lateral gene transfer**
- Prokaryotic cells are **actively exchanging genes in nature**
- HGT “fine-tuning” an organism’s genome to a particular ecological situation or habitat



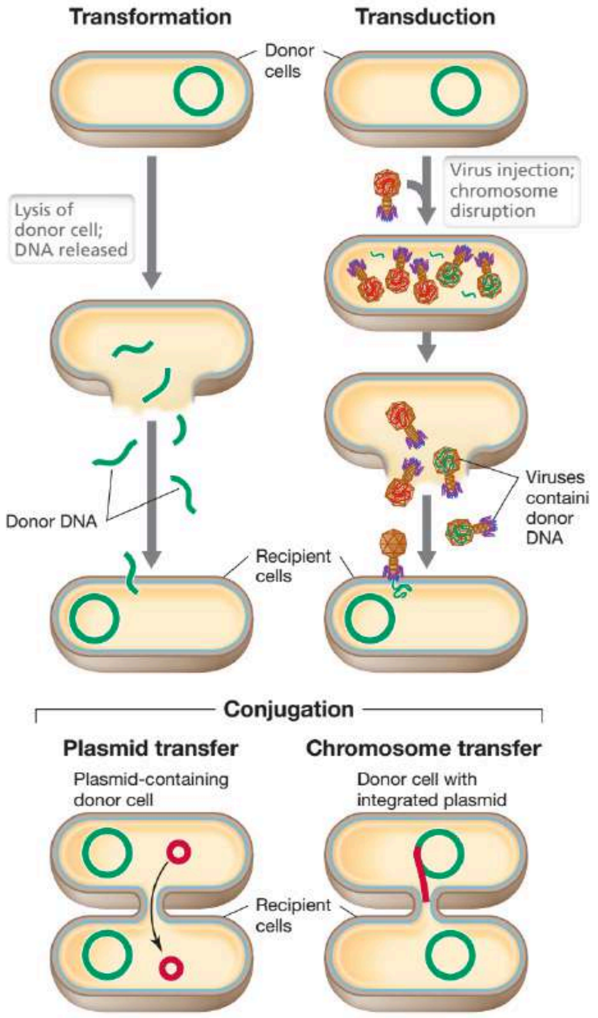
Madigan et al. 2020

- Gene transfer from one cell to another by means other than the vertical process
- In prokaryotic cells, 3 HGT mechanisms: **transformation, transduction, conjugation**
- **HGT** can be detected in genomes once the genes have been annotated:
  a. Presence of genes that encode **proteins** typically found only in **distantly related species**
  b. Presence of a stretch of DNA whose **guanosine/cytosine (GC) content or codon bias** differs significantly from that of the rest of the genome

Horizontal Gene Transfer (HGT), II

1. **Transformation**, in which **free DNA** released from one cell is taken up by another
2. **Transduction**, in which DNA transfer is **mediated by a virus**
3. **Conjugation**, in which DNA transfer requires **cell-to-cell contact** and a conjugative plasmid in the donor cell

- DNA transfer typically occurs in only **one direction: donor —> recipient**



Madigan et al. 2018

- Fate of transferred DNA:
  1. It may be degraded by the recipient cell's **restriction enzymes** or other DNA destruction systems
  2. It may **replicate** by itself (but only if it possesses its own origin of replication, plasmid or phage genome)
  3. It may **recombine** with the recipient cell's chromosome

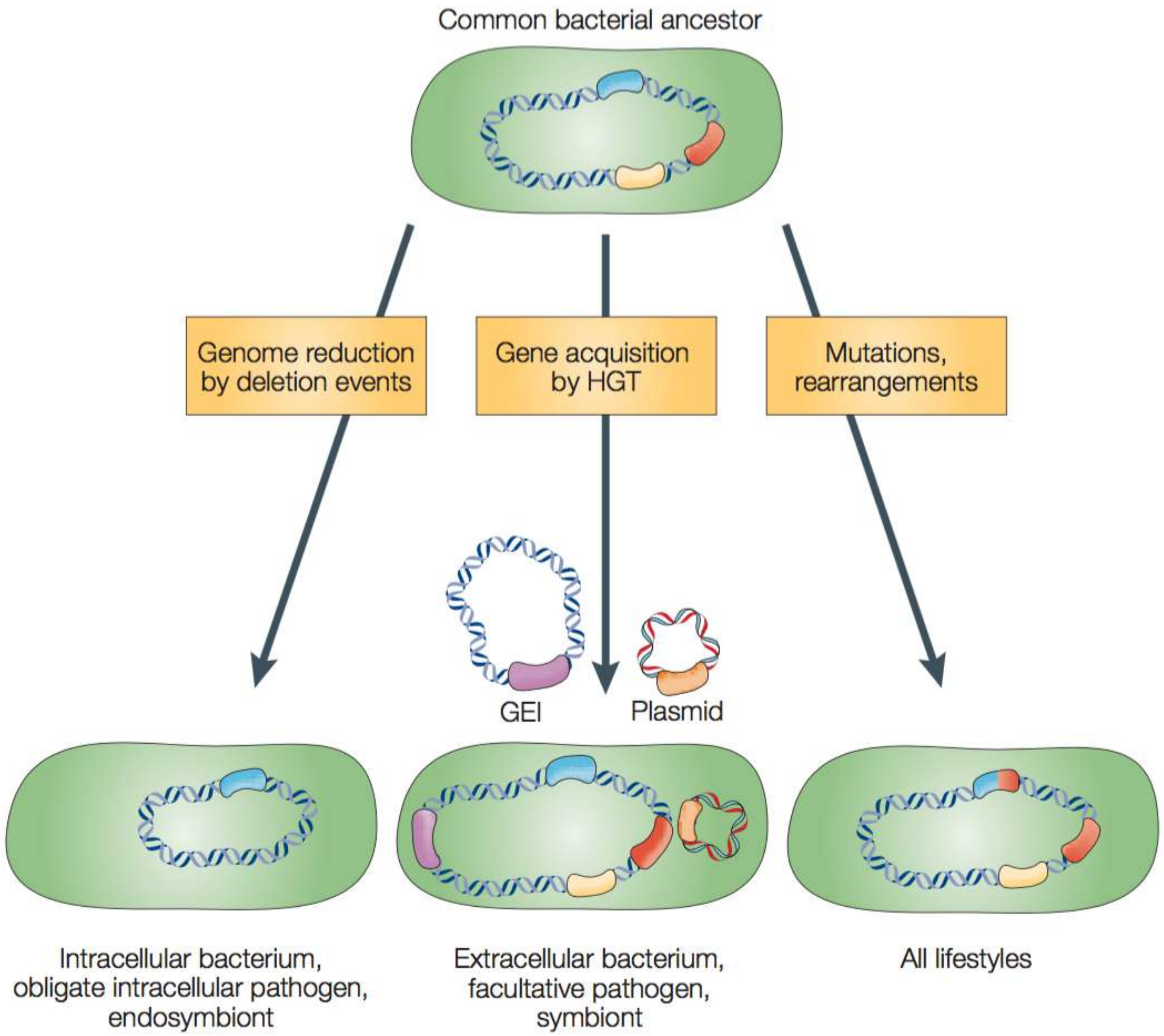
Evolution of bacterial variants by acquisition and loss of genetic information

Genome structure reflects bacterial lifestyle

Genome reduction is common in **intracellular bacteria** (obligate intracellular pathogens, endosymbionts) contributes to the evolution of strictly host-dependent bacterial variants — as bacteria rely on the host cell to compensate for the gene functions that are lost

Gene acquisition by horizontal transfer between different species is common in **extracellular bacteria** (facultative pathogens, symbionts), which involves mobile genetic elements, such as **plasmids**, **genomic islands (GEIs)** and bacteriophages (not shown), increases the versatility and adaptability of the recipient —y allows bacteria to adapt to a new or changing environment

Point mutations and genetic rearrangements constantly contribute to evolution of new gene variants in **all types** of bacteria. HGT, horizontal gene transfer



Common bacterial ancestor

Genome reduction by deletion events

Gene acquisition by HGT

Mutations, rearrangements

GEI

Plasmid

Intracellular bacterium, obligate intracellular pathogen, endosymbiont

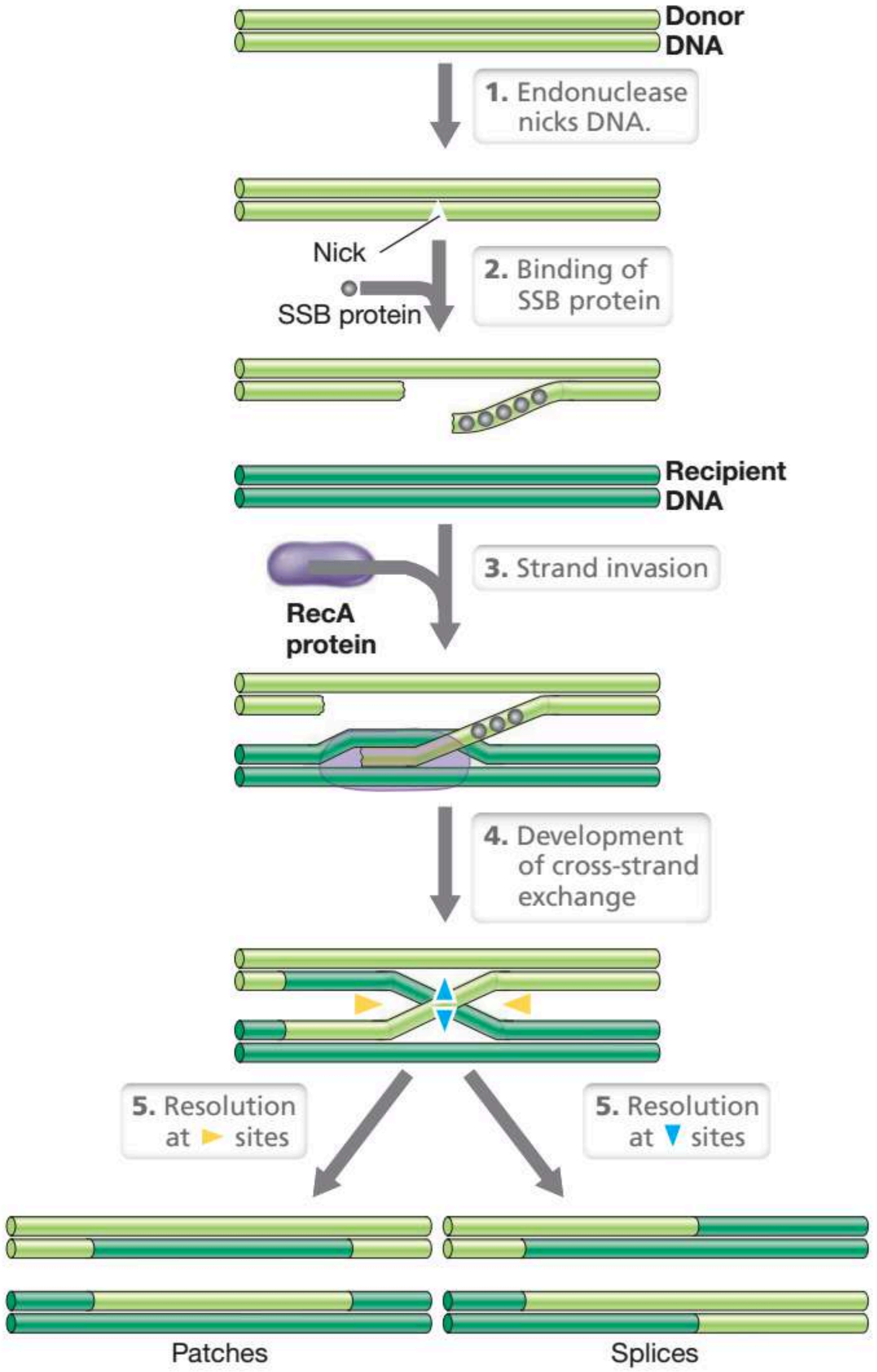
Extracellular bacterium, facultative pathogen, symbiont

All lifestyles

Dobrindt et al., 2004

Recombination, I

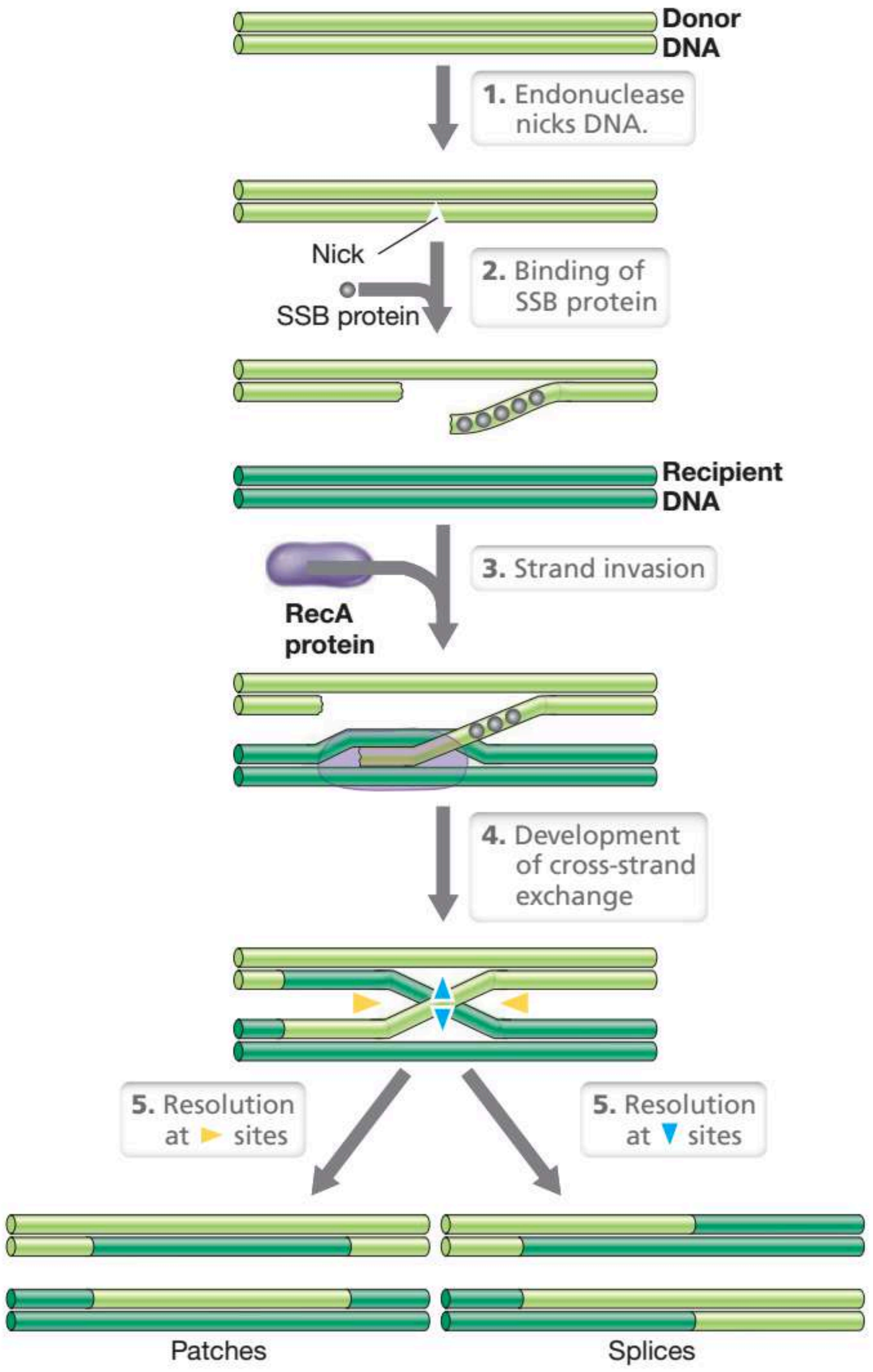
- **Recombination is the physical exchange of DNA** between genetic elements (structures that carry genet information) after HGT
- **Homologous recombination**, a process that results i genetic **exchange between homologous DNA sequences** from 2 different sources
- **Homologous DNA** sequences are those that have nearly the **same sequence**; therefore, bases can pair over an extended length of the two DNA molecules to facilitate exchange
- **RecA protein**, SOS repair system is the key to **homologous recombination**
- RecA is essential in nearly every homologous recombination pathway



Madigan et al. 2018

Recombination, II

1. **Endonuclease cuts DNA in the middle of a strand —> nicking one strand of the donor DNA molecule**
2. Nicked strand is separated from the other strand by proteins with **helicase activity and binds single-strand binding protein + RecA**
3. **Base pairing with the complementary sequence in the recipient DNA molecule —> displaces the othe strand of the recipient DNA molecule (strand invasion)**
4. Heteroduplex according to spatial orientation: patches splices



Madigan et al. 2018

Transformation

- Genetic transfer process by which **free DNA is incorporated** into a recipient cell and brings about genetic change
- Several organisms are **naturally transformable**
- Because the DNA in prokaryotic cells is present as a large single molecule, when a cell is gently lysed, its DNA pours out
- **Bacterial chromosomes break easily because of their extreme length** (if linearized, the *Bacillus subtilis* chromosome would be 1700 µm long, ~4.2 Mb, fragment of 10 kb ~ 1000 bases per genes —> 10 genes)
- A single cell incorporates only one or at most a few DNA fragments, so **only a small proportion of the genes** of one cell can be transferred to another in a single transformation event

Competence, I

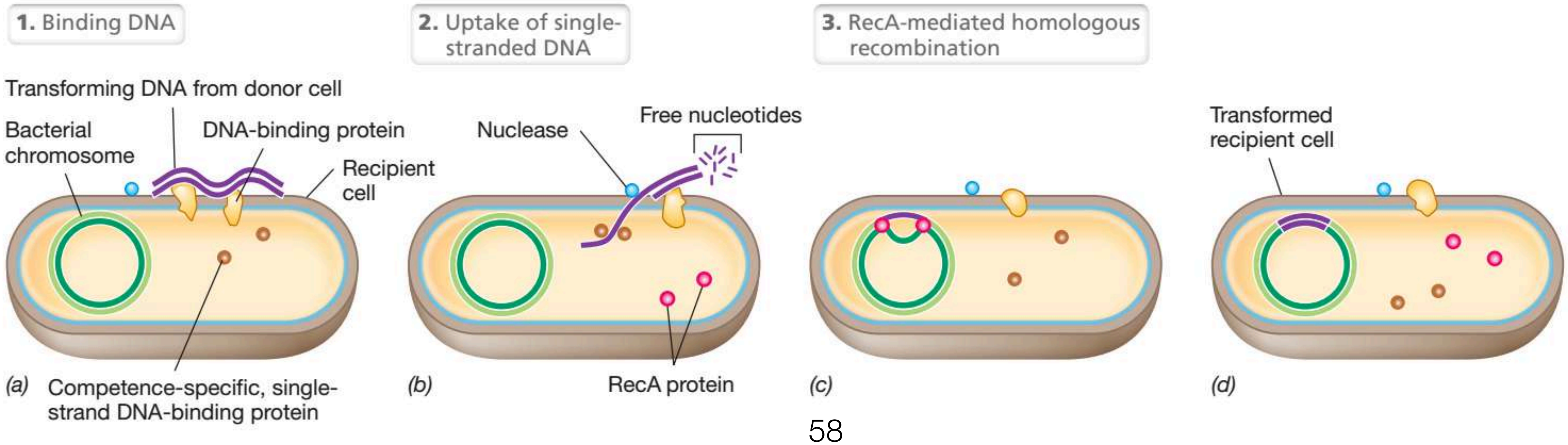
- **Ability of a cell to take up DNA and be transformed, is genetically determined**
- Competence is regulated: special proteins play a role in **DNA uptake and processing**
- **Competence-specific proteins**: membrane associated DNA-binding protein, a cell wall autolysin, and various nucleases
- Natural competence in ***B. subtilis*** is regulated by ***quorum sensing*** (cell abundance behavior), only 20% of population are *competent for hours*
- Cells produce and excrete a small peptide during growth, and the accumulation of this peptide to high concentrations **induces the cells to become competent**
- In ***Streptococcus***, 100% of the cells can become competent, but only for *a brief period during the growt*h cycle

Competence, II

- Competence in ***V. cholerae*** is controlled not only by quorum sensing but also by chitin sensing and catabolite repression
- *Acinetobacter, Bacillus, Streptococcus, Haemophilus, Neisseria,* and *Thermus* **are naturally competent** and easy to transform **others are not**.
- Natural competence provides a nutritional advantage, **as free DNA is rich in carbon, nitrogen, and phosphorus**
- If *E. coli* are treated with **high concentrations of Ca^{2+} and then chilled** —> competent for dsDNA
- *Electroporation* is a physical technique that is used to get DNA into organisms that are difficult or impossible to transform, especially cells that contain thick cell walls —> **exposure to brief, high-voltage electrical pulses—> cell envelope permeable**
- *During natural transformation competent bacteria* ***reversibly bind DNA —> binding irreversible***

Competence, III

- *Streptococcus pneumoniae* (the cause of bacterial pneumonia) cell can bind ~ 10 ds DNA of 10–15 kbp each
- ds-fragments are taken up —> ssDNA (~8 kb)
- DNA fragments in the mixture compete with each other for uptake and thus the probability of a transformant taking up DNA that **confers an advantage or a selectable marker decreases**
- During transformation, DNA is bound at the cell surface by a DNA-binding protein resembles a pilus that is able to pull the DNA into the periplasm of a gram-negative bacterium or through the thick cell wall of a gram-positive bacterium
- Competence-specific protein binds the donor DNA, for protection —> **RecA** —> **integration into recipient genome**

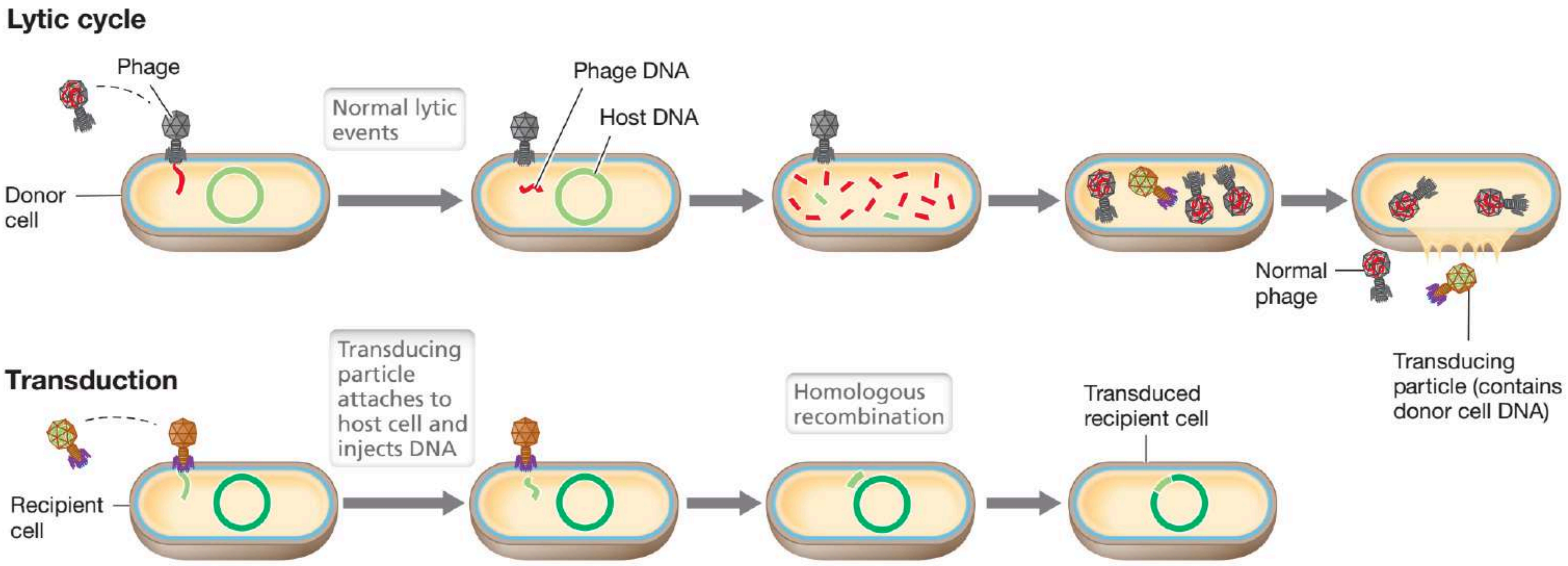


Madigan et al. 2020

Transduction, I

- A bacterial virus (bacteriophage) transfers DNA from one cell to another
- **Generalized transduction**, DNA derived from virtually **any portion of the host** genome is packaged inside the mature virion in place of the virus genome —> need for recombination recipient bacterial chromosome
- **Specialized transduction**, DNA from a specific region of the host chromosome is integrated directly into the virus genome—usually replacing some of the virus genes (temperate viruses) —> integration into host chromosome
- **Not all phages can transduce and not all bacteria are transducible**
- Bacteriophages ~10X prokaryotic —> transduction likely plays an important role in gene transfer in the environment
- Example: Multiple antibiotic-resistance genes among strains of *Salmonella enterica* (*typhimurium*), Shiga-like toxin genes in *Escherichia coli*, virulence factors in *Vibrio cholerae*, and genes encoding photosynthetic proteins in cyanobacteria

Transduction, II

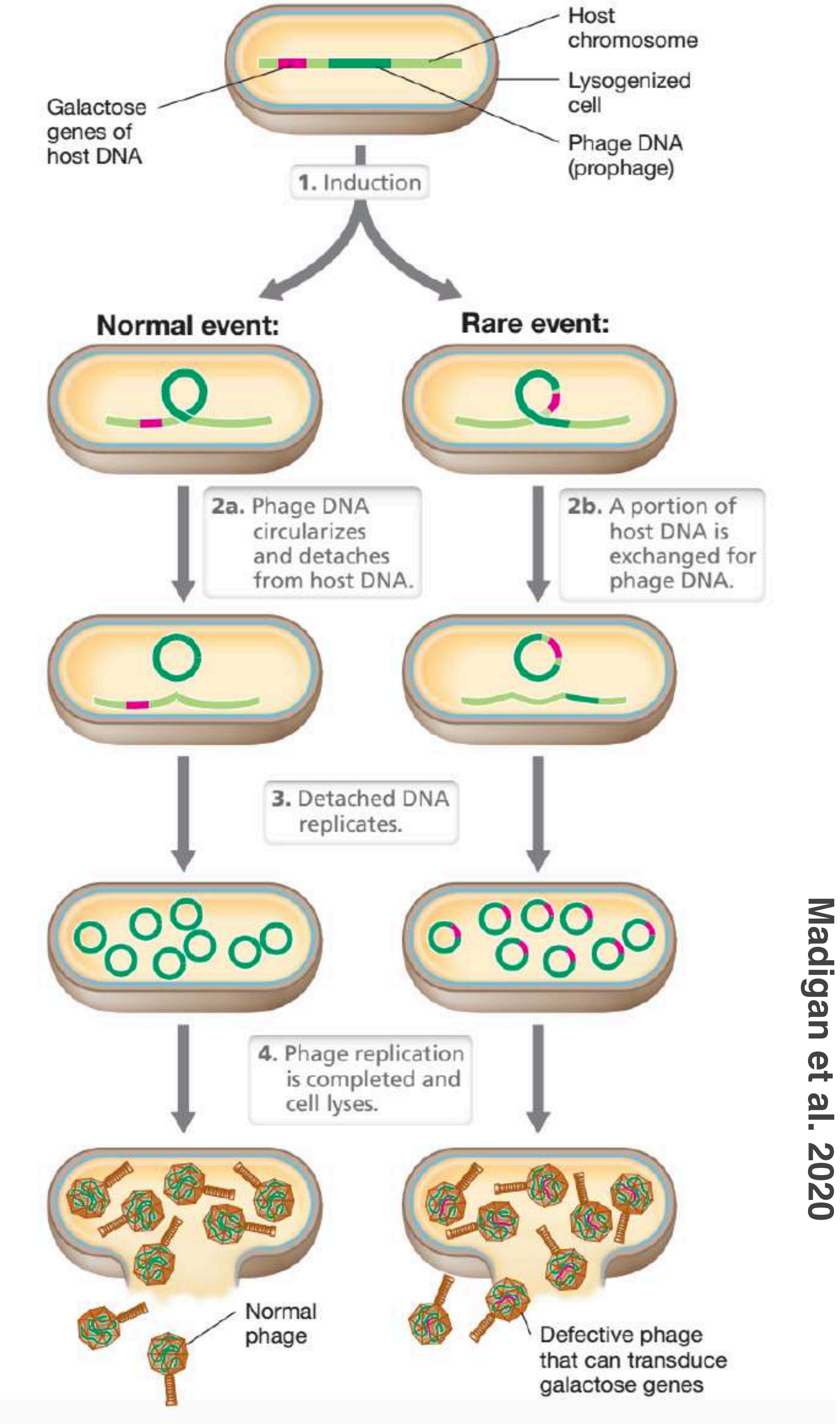


Madigan et al. 2020

- **Generalized transduction: any gene on the donor chromosome can be transferred to the recipient: transductant**
- *S. enterica* with phage P22 and with phage P1 in *E. coli*
- Lytic cycle: the enzymes responsible for packaging viral DNA into the bacteriophage sometimes package host DNA accidentally, **transducing particle cannot lead to viral lytic infection**
- Upon lysis of the cell, **transducing particles are released along with normal virions** that contain the virus genome
- During following infections a small proportion of the population receives transducing particles that inject the DNA they packaged from the previous host bacterium
- DNA cannot replicate but it can **recombine with the DNA of the new host**, small # of defective particles low probability transduction for any given gene (low frequency)

Transduction, III

- **Specialized transduction allows extremely efficient and selective transfers only a small region chromosome**
- Galactose catabolism genes were transduced by the temperate phage lambda of *E. coli*
- **Phage genome is excised incorrectly** —> some adjacent genes to one side of the prophage (the galactose operon) are excised along with phage DNA
- For a lambda virion to be infectious, **there is a limit to the amount of phage DNA that can be replaced with host DNA**
- **If a helper phage is used together with a defective phage** in a mixed infection, then far fewer phage-specific genes are needed in the defective phage: **att (attachment) region, the cos site (cohesive ends, for packaging), and the replication origin of the lambda genome**
- *Alteration of the phenotype of a host cell by lysogenization* is called **phage conversion**



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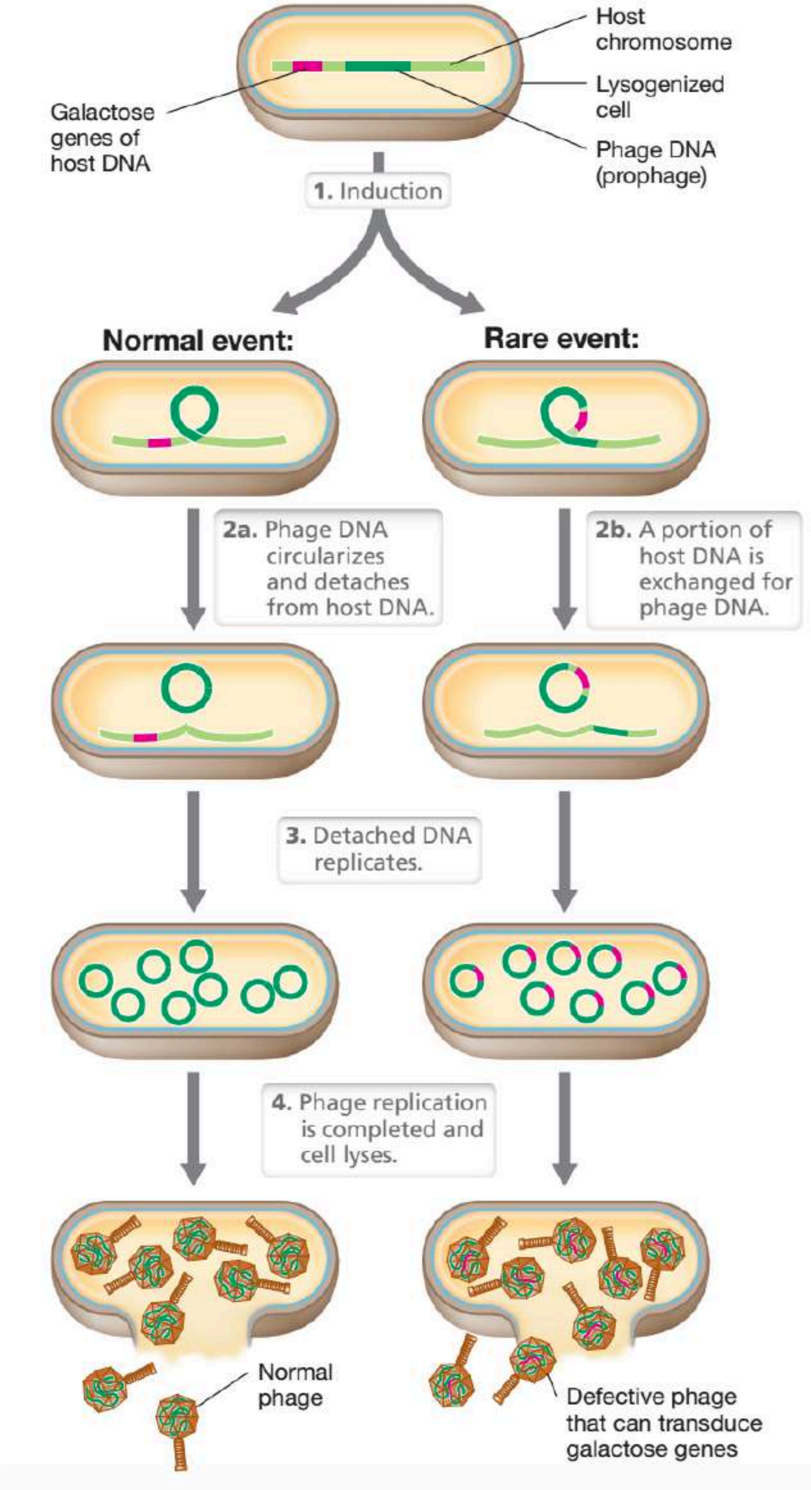
Transduction, IV

- **When a normal (that is, non defective) temperate phage lysogenizes a cell and becomes a prophage**, the cell becomes immune to further infection by the same type of phage —> **change in phenotype**
- However, other phenotypic changes unrelated to phage immunity are often observed in phage conversion of lysogenized cells:

1. Change in structure of a polysaccharide on the cell surface of *S. enterica (anatum)* w. bacteriophage ε15
2. Conversion of non-toxinproducing strains of *Corynebacterium diphtheriae* (cause of disease diphtheria) to toxin-producing (pathogenic) strains w. bacteriophage b

- Lysogeny likely carries **strong selective value for the host cell b/c confers resistance to infection by viruses of the same type**
- **Phage conversion** of evolutionary significance **b/c results in genetic alteration of host cells**



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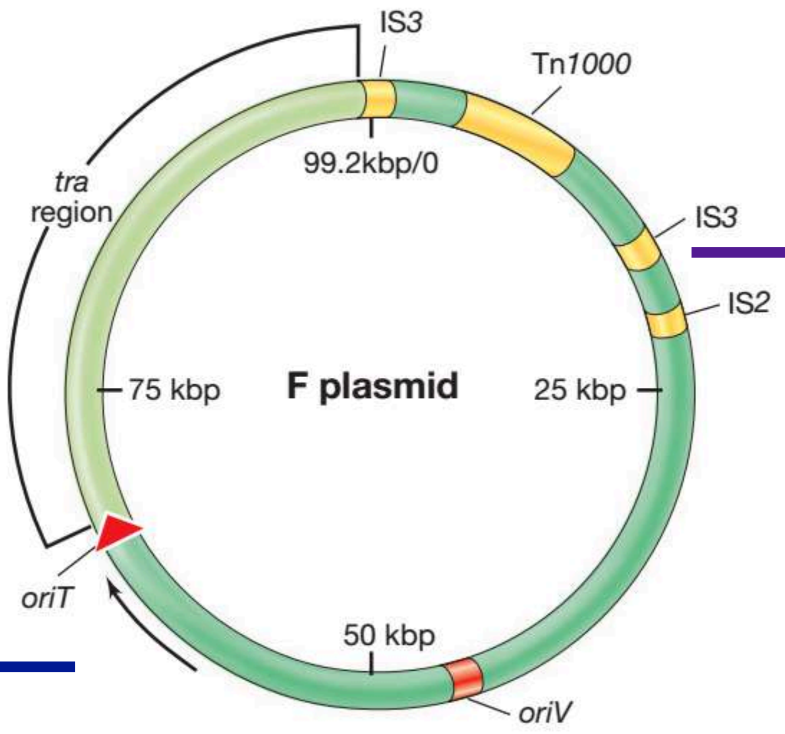
Conjugation, I



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Recombination

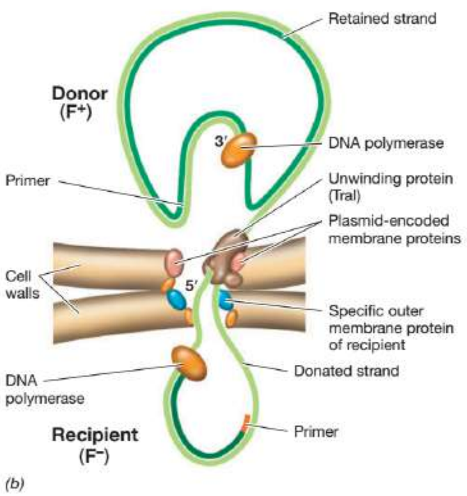
DNA transfer starts at oriT (origin of transfer) site

F plasmid

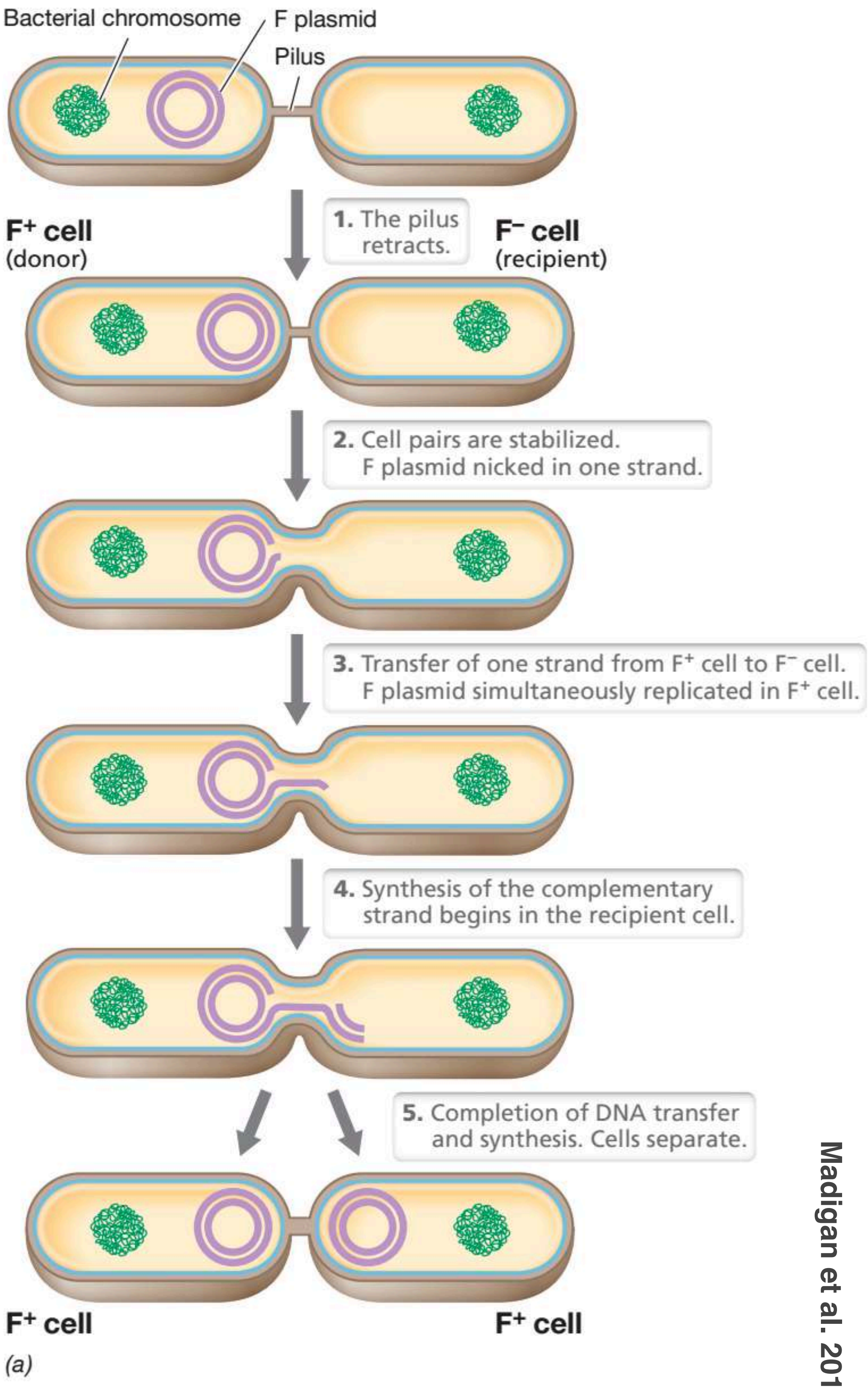
- Conjugation requires **cell-to-cell contact** (mating), plasmid encoded
- **Conjugative plasmids** use this mechanism to **transfer copies of themselves** and the **genes** they encode (e.g. antibiotic resistance), to new host cells
- Conjugation requires a **donor** cell, which contains the conjugative plasmid, and a **recipient** cell, which does not
- Conjugation can transfer other **plasmids or the host chromosome itself**
- *E. coli* F plasmid can mobilize the host chromosome
- F plasmid (F stands for “fertility”) is circular DNA molecule of 99,159 bp, genes that regulate DNA replication and transposable elements for chromosome *integration/insertion (IS3)* into the host chromosome, *tra region (with genes that encode transfer functions)*, *sex pilus, type IV secretion system* to transfer DNA (donor only)

Conjugation, II

F plasmid



- *Pilus makes specific contact with a receptor on the recipient cell* and then is retracted by disassembling its subunits—> cells come together
- Donor and recipient cells remain in *contact by binding coupling proteins* located in outer membrane
- *DNA is transferred from donor (F+) to recipient (F-)* cell through this conjugation junction
- DNA is synthesized by *rolling circle replication*
- DNA transfer is triggered by cell-to-cell contact, at which time *one strand of the circular plasmid DNA is nicked by nicking enzyme TraI and is transferred to the recipient*
- *TraI has helicase activity to unwind the strand to be transferred*
- As this transfer occurs, DNA synthesis by the rolling circle mechanism replaces the transferred strand in the donor, while a complementary DNA strand is being made in the recipient —> complete plasmids in donor and recipient
- *~ 100 kbp in 5 min, spread rapidly ~ infectious agents*



1. The pilus retracts.

2. Cell pairs are stabilized. F plasmid nicked in one strand.

3. Transfer of one strand from F⁺ cell to F⁻ cell. F plasmid simultaneously replicated in F⁺ cell.

4. Synthesis of the complementary strand begins in the recipient cell.

5. Completion of DNA transfer and synthesis. Cells separate.

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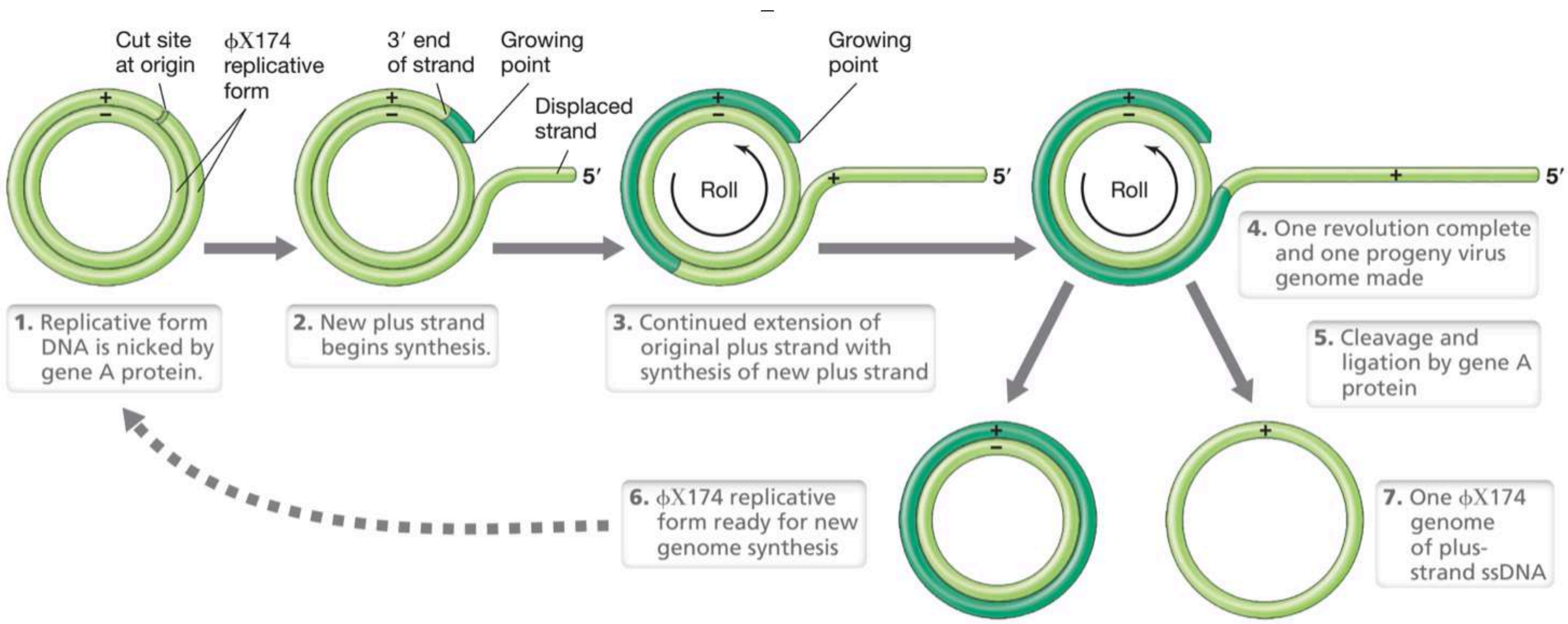
Rolling circle replication

- Not a Semiconservative replication ***only one strand is transcribed, off of negative strand* of the replicative form in fX174**
- In the synthesin of the fX174 genome, the rolling circle facilitates the *continuous production* of positive strands from the replicative form
- The positive strand of the latter is nicked by A protein and the **3' end of the exposed DNA** is used to prime synthesis of a new strand
- **Only the negative strand serves as a template**
- When the growing viral strand reaches unit length (5386 residues for fX174), **the A protein cleaves it and then ligates the two ends of the newly synthesized single strand to give a ssDNA circle**



1. Replicative form DNA is nicked by gene A protein.

2. New plus strand begins synthesis.

3. Continued extension of original plus strand with synthesis of new plus strand

4. One revolution complete and one progeny virus genome made

5. Cleavage and ligation by gene A protein

6. ϕX174 replicative form ready for new genome synthesis

7. One ϕX174 genome of plus-strand ssDNA

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Conjugation, III

- Chromosome mobilization by plasmid-mediated conjugation
- **F plasmid is an episome, a plasmid that can integrate into the host chromosome**
- When F plasmid is integrated, chromosomal genes can be transferred along with the plasmid
- Following genetic recombination between donor and recipient DNA, horizontal transfer of chromosomal genes by this mechanism can be extensive
- **F+ cells nonintegrated F plasmid** vs **Hfr cells (for high frequency of recombination) integrated into the chromosome**
- **Rolling circle replication** is initiated by F plasmid, replication **continues on into the chromosome**

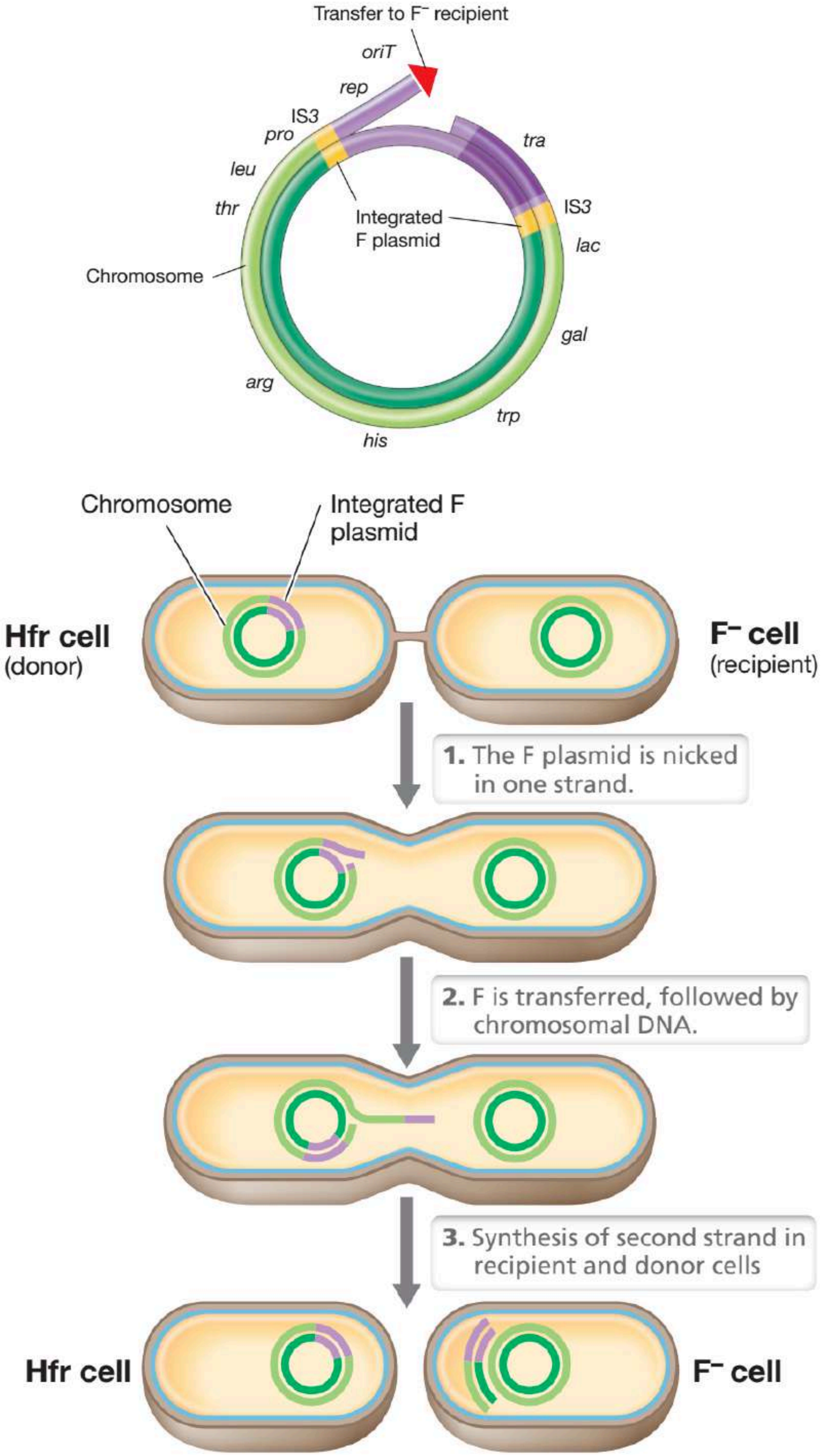
(1) the ability to synthesize the **F pilus**

(2) the **mobilization of DNA** for transfer to another cell

(3) the **alteration of surface receptors** —> cell **unable to take up a second copy F** plasmid or any genetically related plasmids

Conjugation, IV

- F plasmid & *E.coli* chromosome carry several copies of mobile genetic elements called **insertion sequences (IS)**
- **IS regions of sequence homology between chromosomal and F plasmid DNA**—> homologous recombination —> diverse Hfr (given the place of insertion in chromosome and direction)
- When a recipient cell is encountered, conjugation is triggered
- DNA strand usually breaks during transfer, only part of the donor chromosome is typically transferred
- Recipient does not become Hfr (or F+) because only **part of the integrated F plasmid is transferred**
- **F- only if recombined w. chromosome can express new phenotypes**



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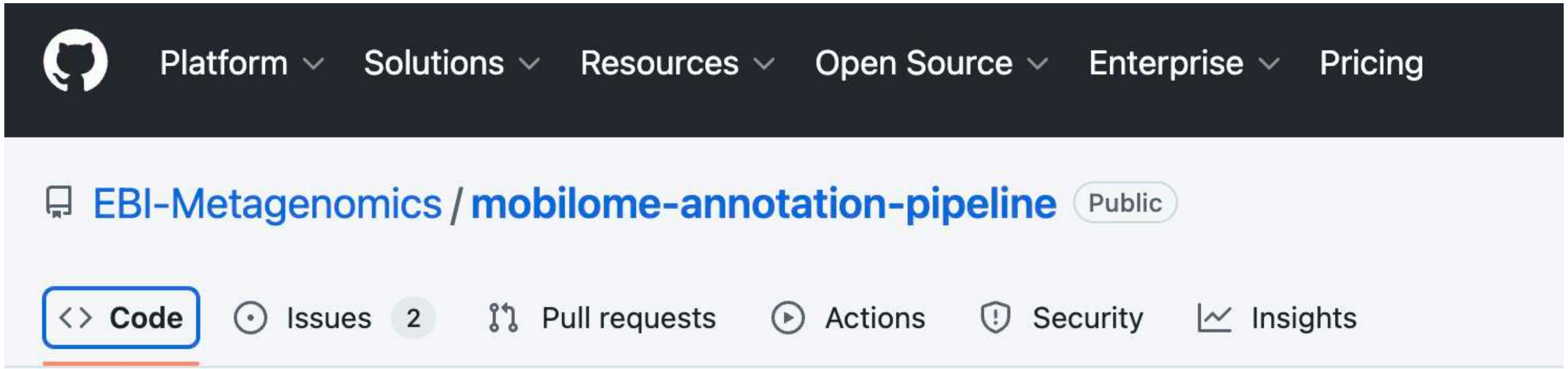
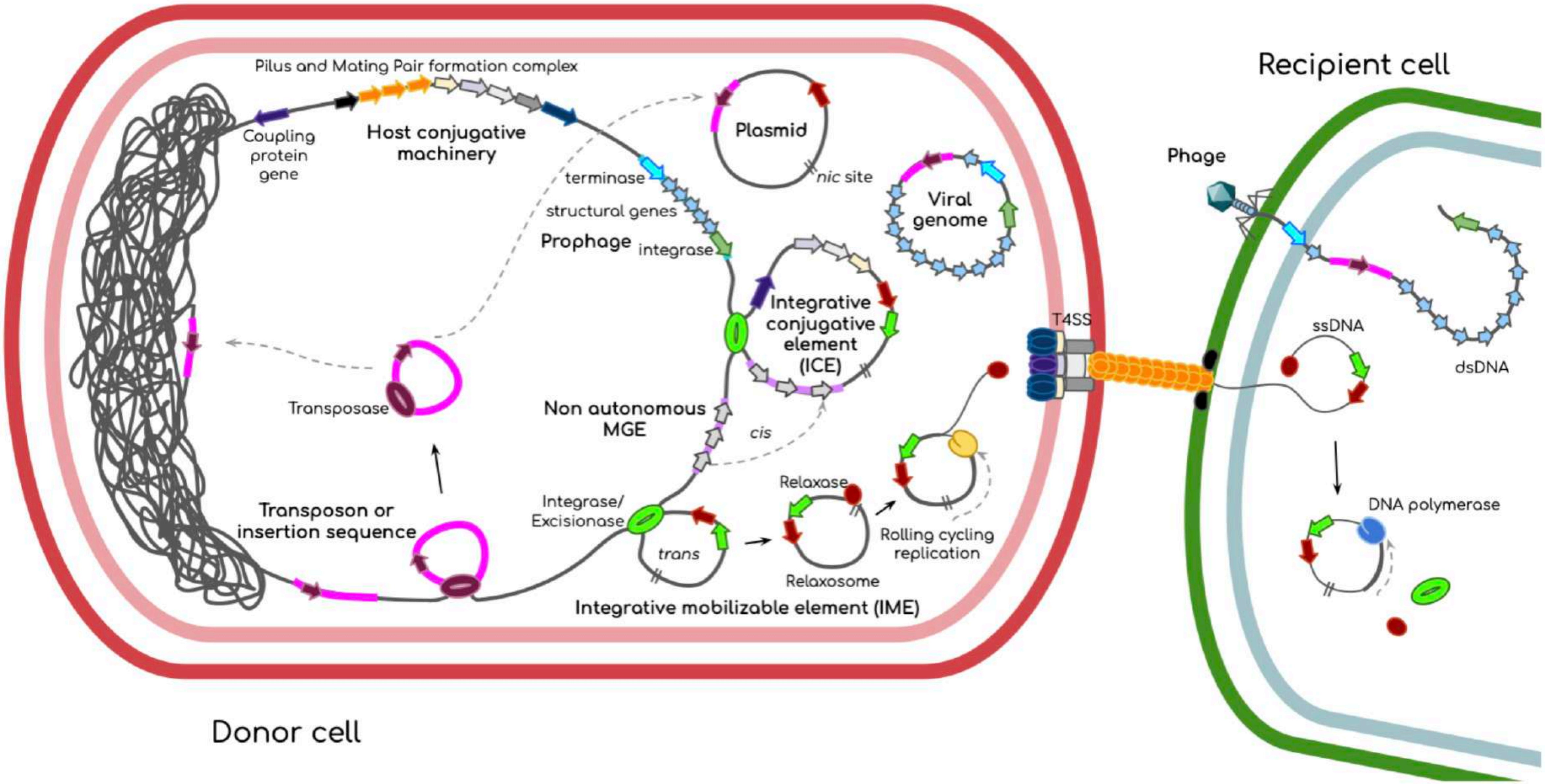


EBI-Metagenomics / **mobilome-annotation-pipeline**



https://github.com/EBI-Metagenomics/mobilome-annotation-pipeline