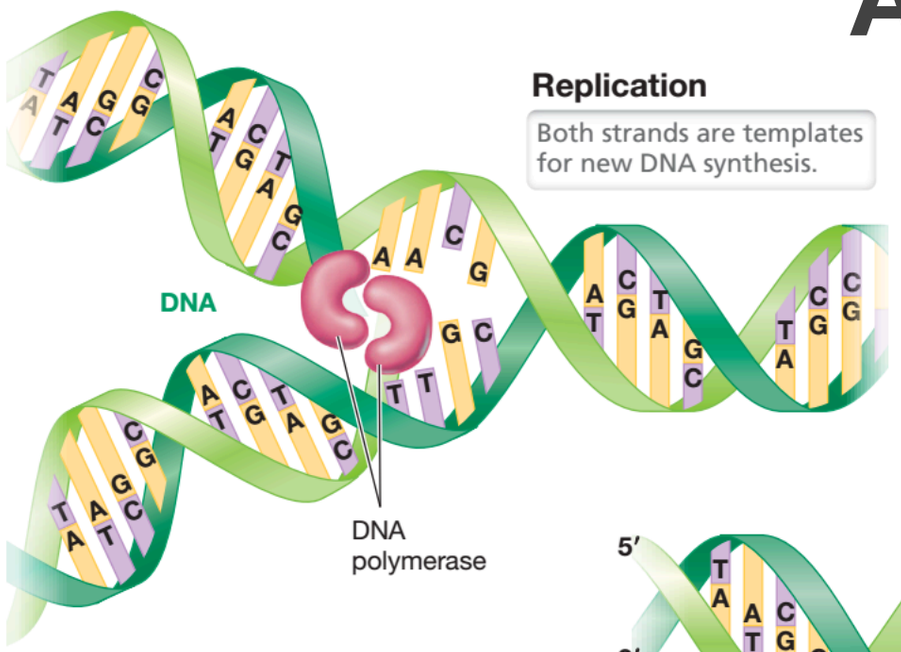
212SM_L05b

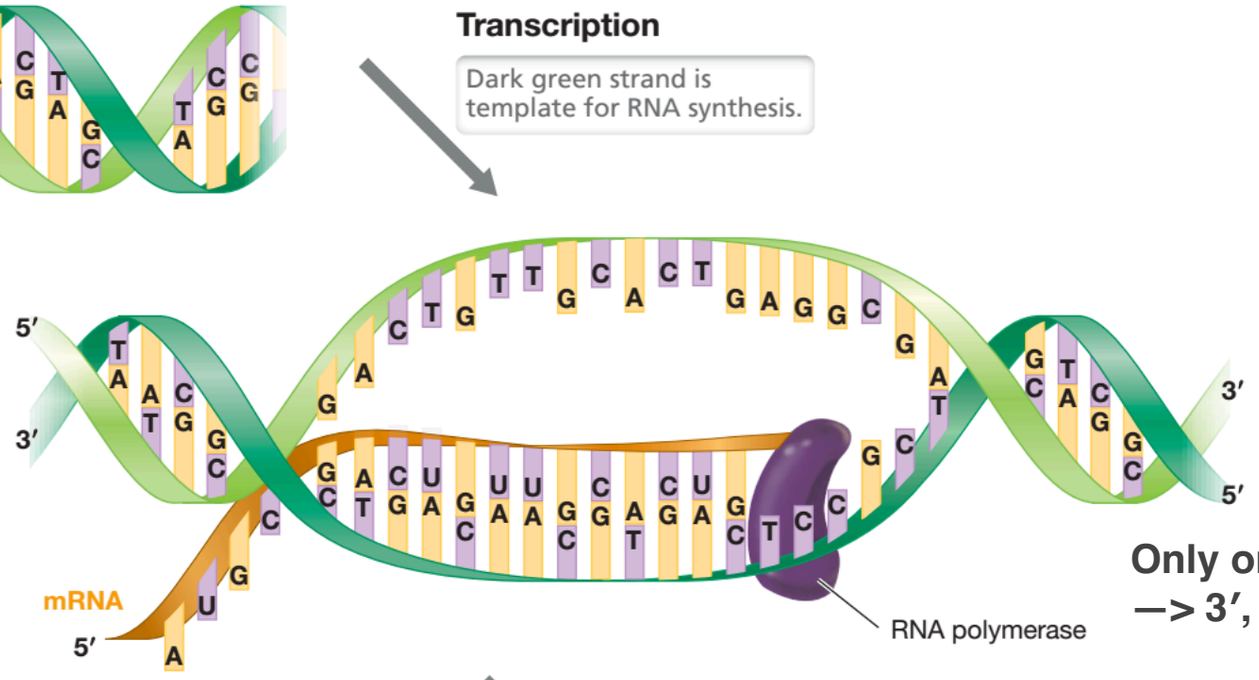
Microbial chemical weapons

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	$2–4 \times 10^6$		
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 ($D \approx 10\ \mu m^2/s$)	~0.01 s	~0.2 s	~1–10 s
diffusion time of small molecule across cell ($D \approx 100\ \mu m^2/s$)	~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		

Replication, Transcription, Translation & Antibiotics

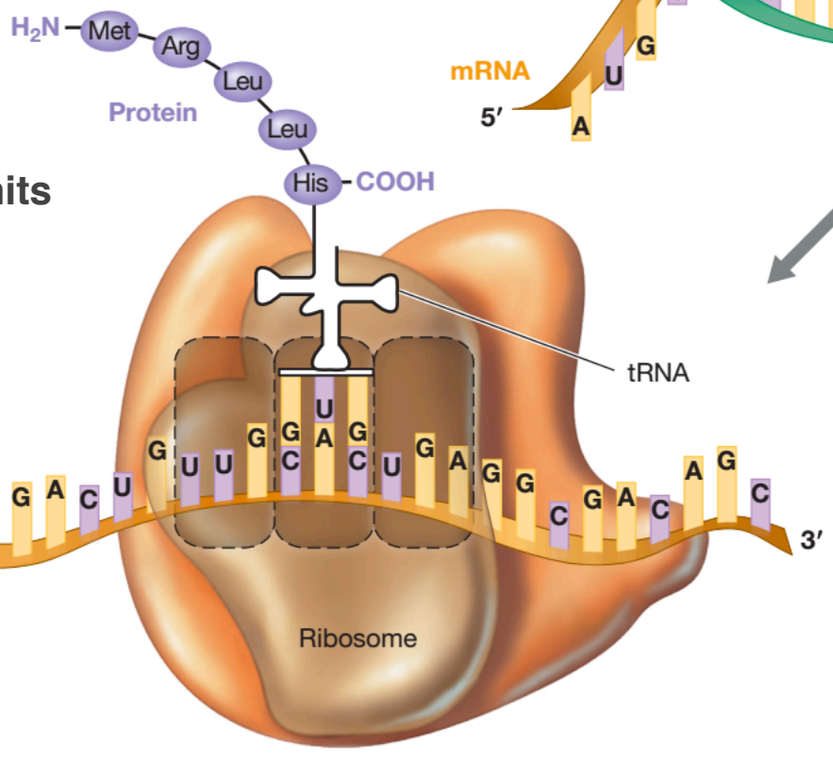


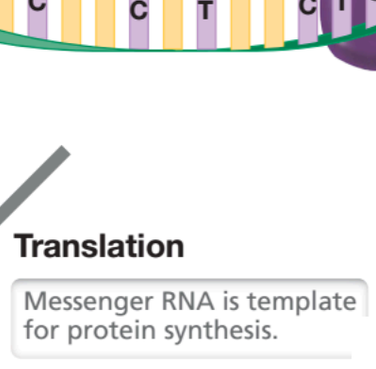
Replication always 5′—> 3′, adding a new nucleotide to the 3′-OH of the growing chain, RNA primer

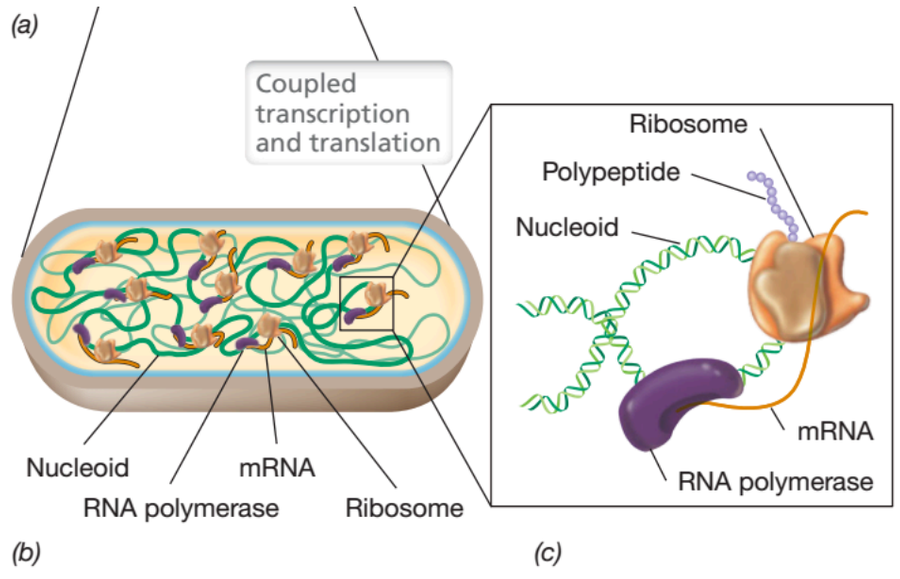


Only one chain growth is 5′ —> 3′, no priming

Ribosome subunits alternately associate and dissociate





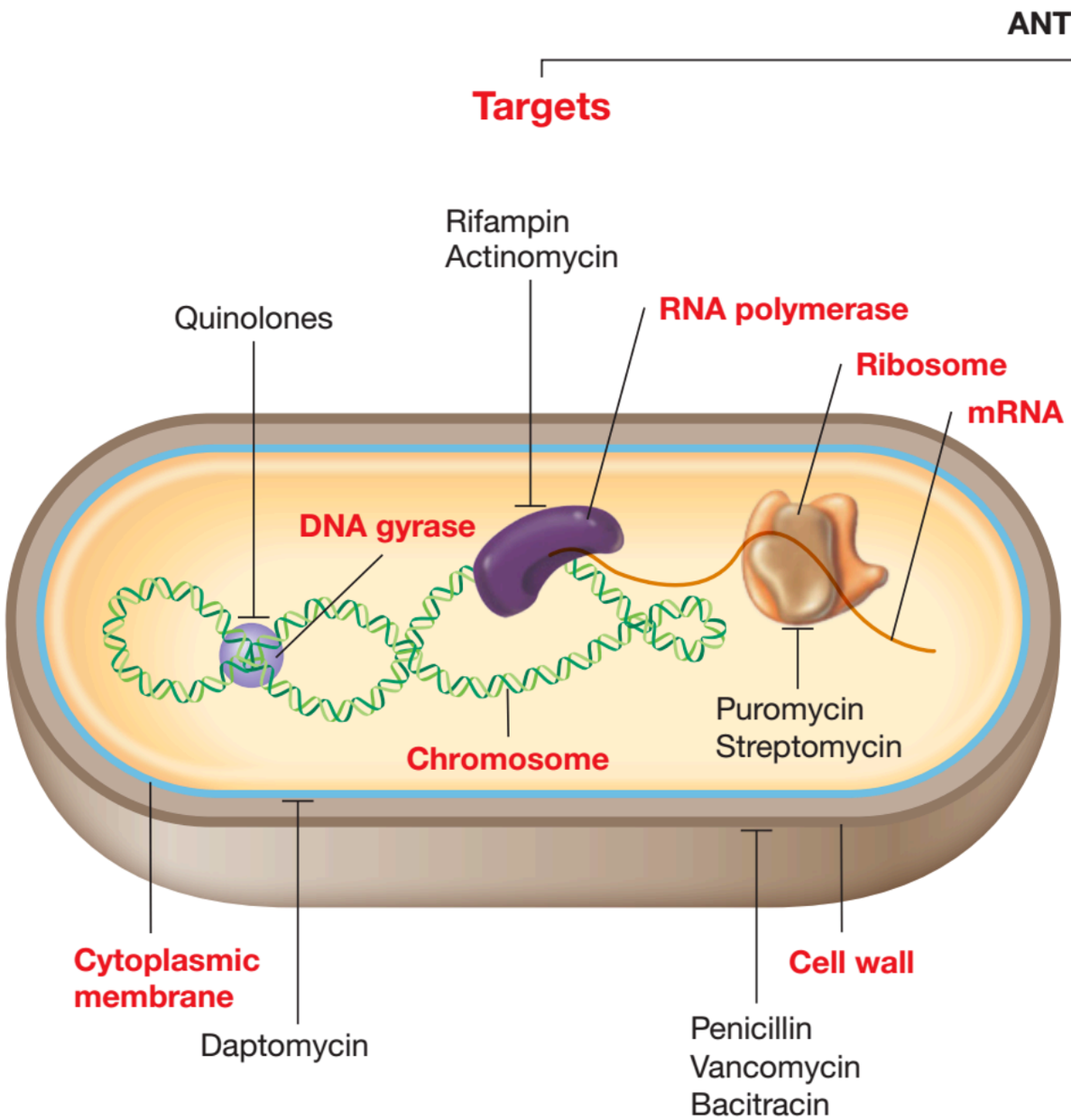


Ribosome-binding site at 5′ end of mRNA, RBS is complementary at 3′ end of the 16S rRNA part

Madigan et al. 2020

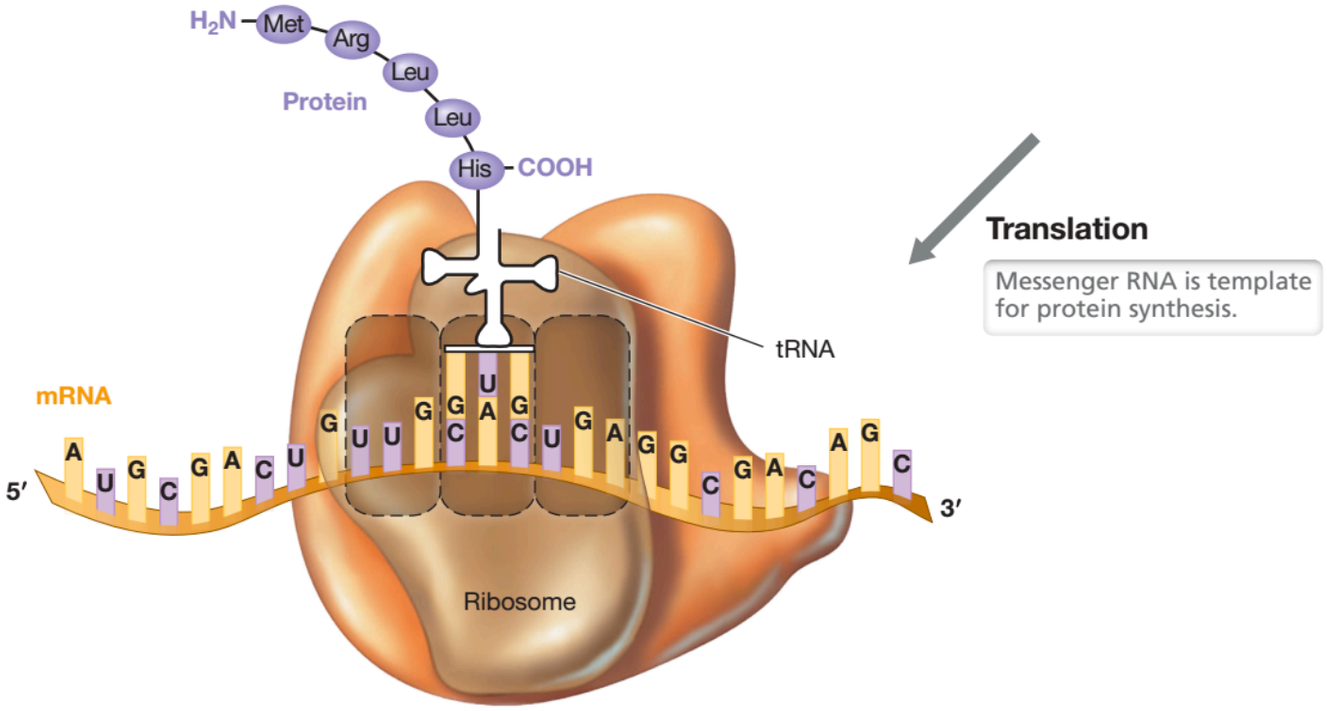
ANTIBIOTICS I

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



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**

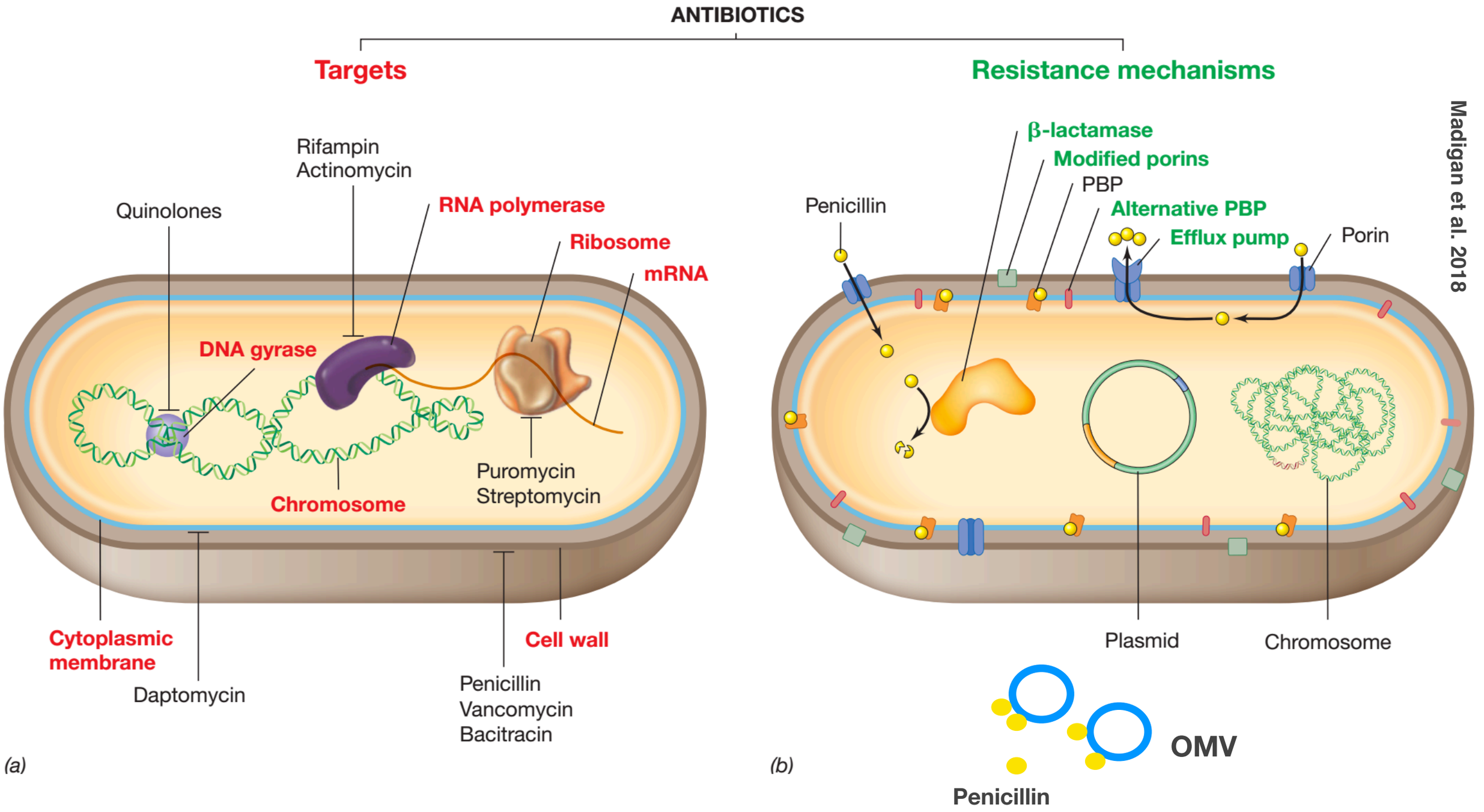


Madigan et al. 2020

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



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**)

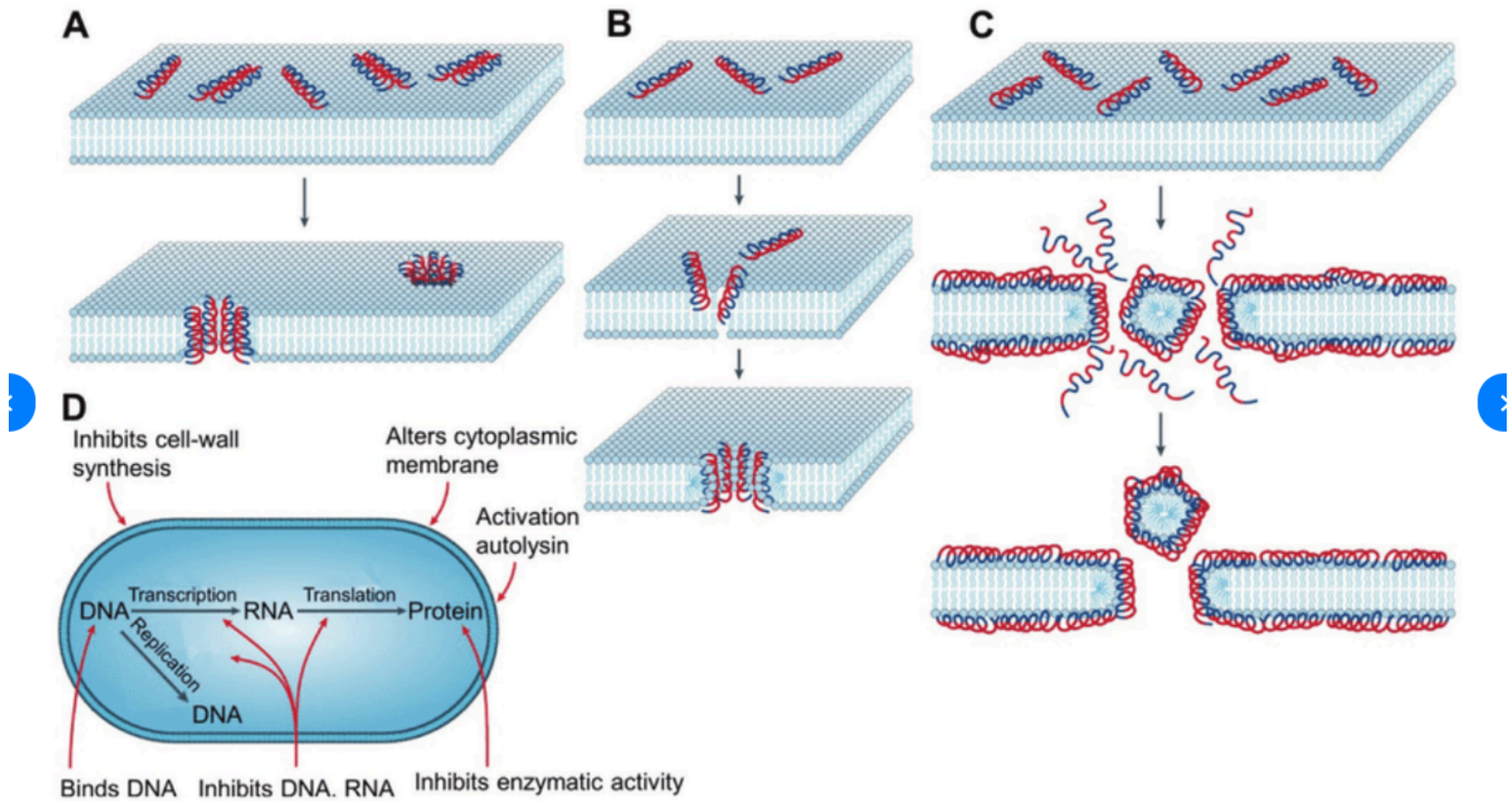
- **Vancomycin** inhibits **peptidoglycan synthesis** in gram positive bacteria by **binding to pentapeptide of peptidoglycan precursors and preventing formation of peptide interbridges by transpeptidases**

- **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

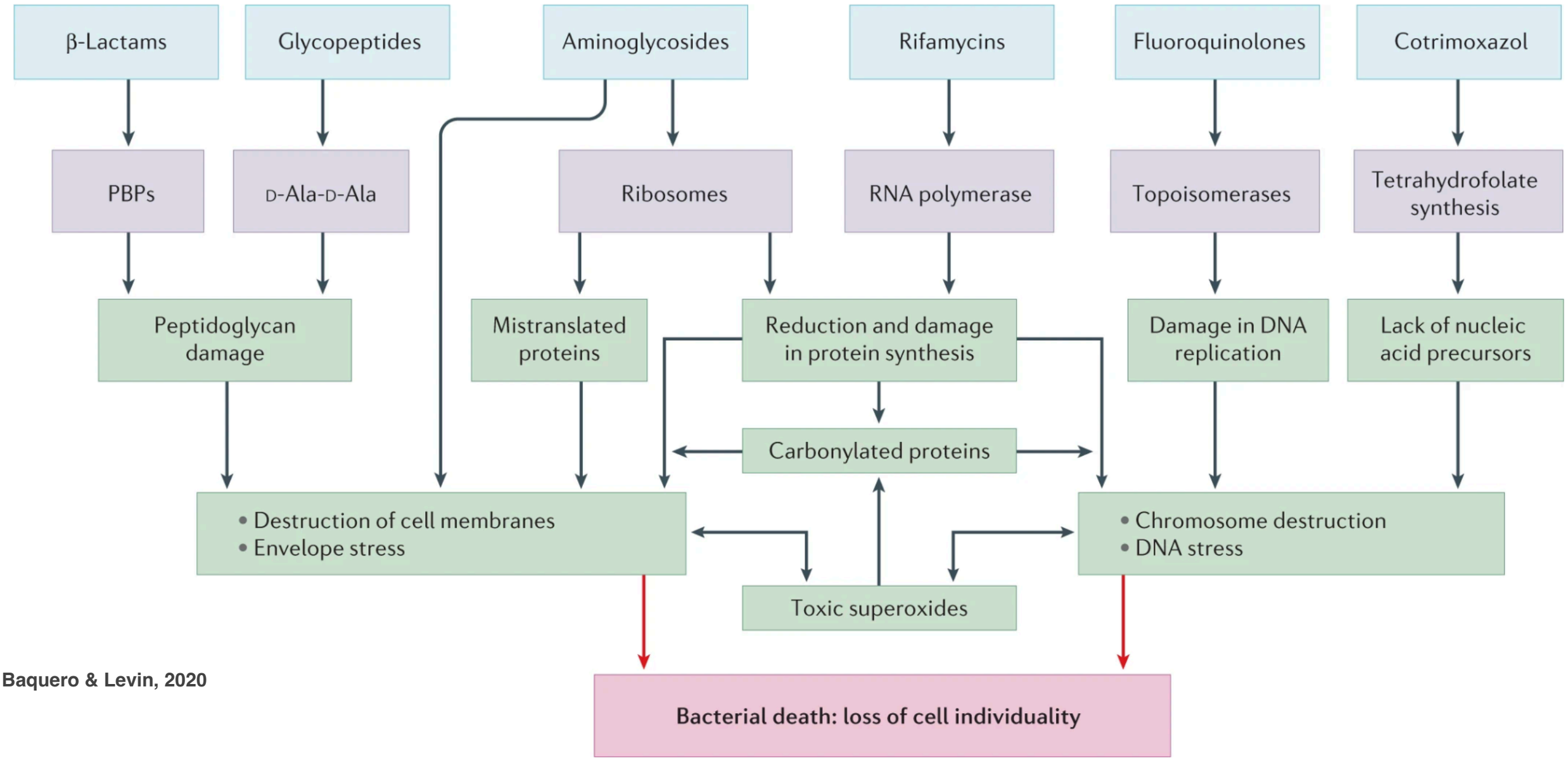
- **Antimicrobial peptides** (AMPs) are **oligopeptides** with a varying number (from five to over a hundred) of amino acids
- AMPs have a **broad spectrum** of targeted organisms ranging from viruses, bacteria, fungi to parasites
- Historically AMPs have also been referred to as cationic host defense 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



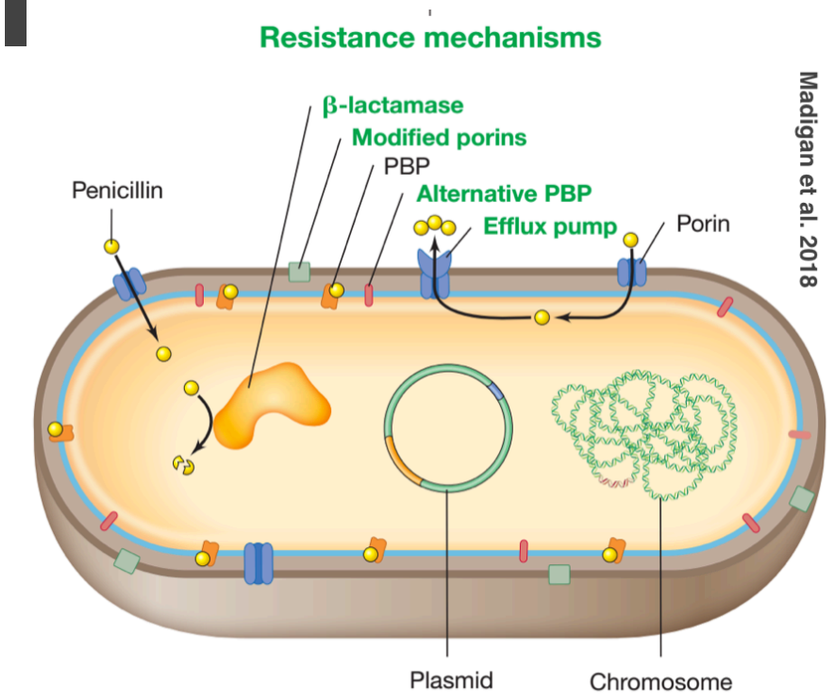
Baquero & Levin, 2020

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).

Antibiotic Resistance, I

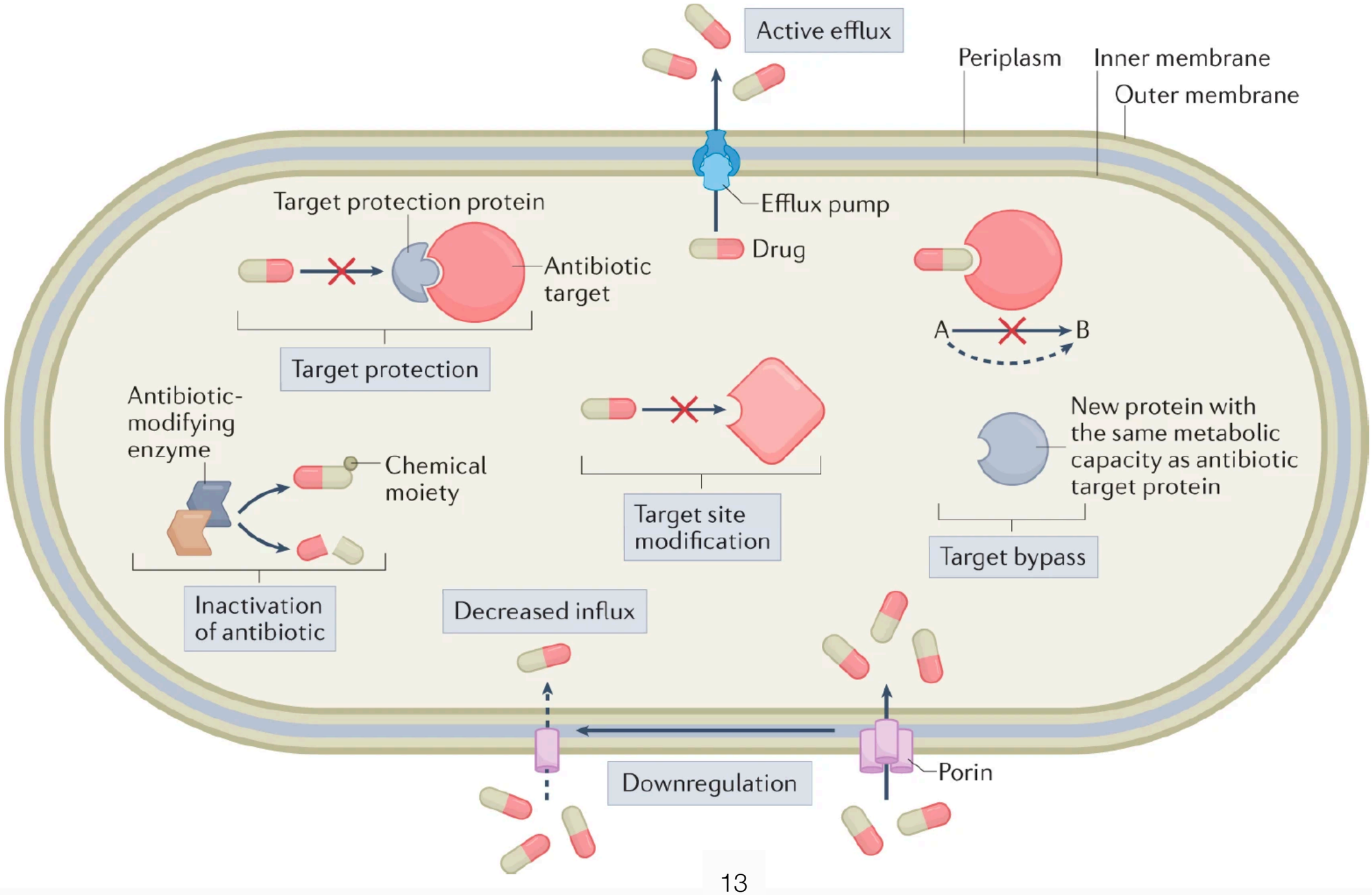
- Resistance mechanisms:

    1. **Modification of the drug target**
    2. **Enzymatic inactivation**
    3. **Removal from the cell via efflux pumps**
    4. **Metabolic bypasses**

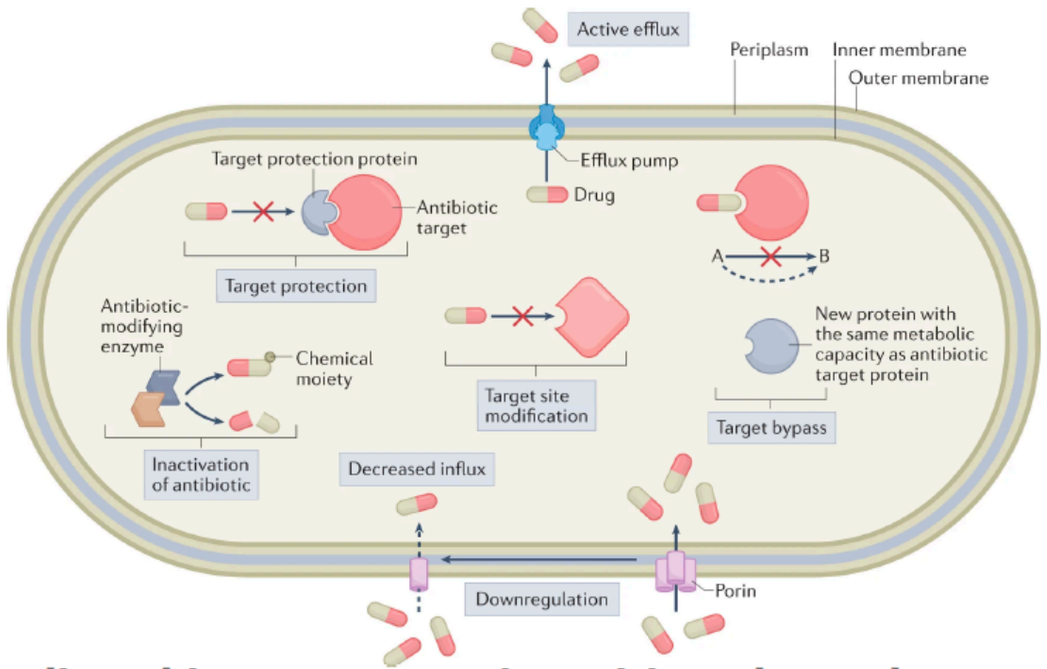


- **Random mutation** can lead to antibiotic resistance
- Resistance genes can also exist on a variety of **mobile genetic elements** and such genes can be readily transmitted between bacteria of same or different species by horizontal gene flow
- Many **mobile resistance genes, on R plasmids**, encode 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

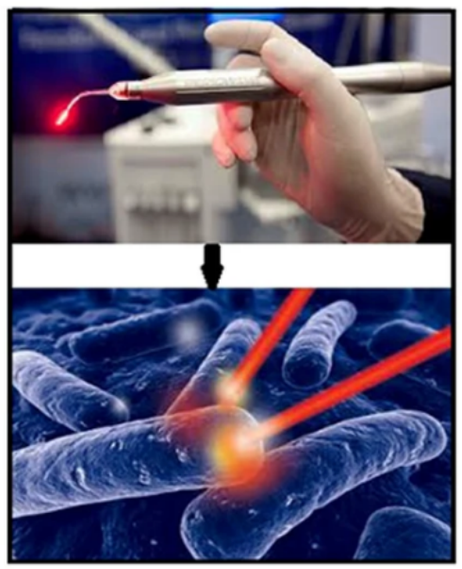
Antibiotic Resistance I

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**

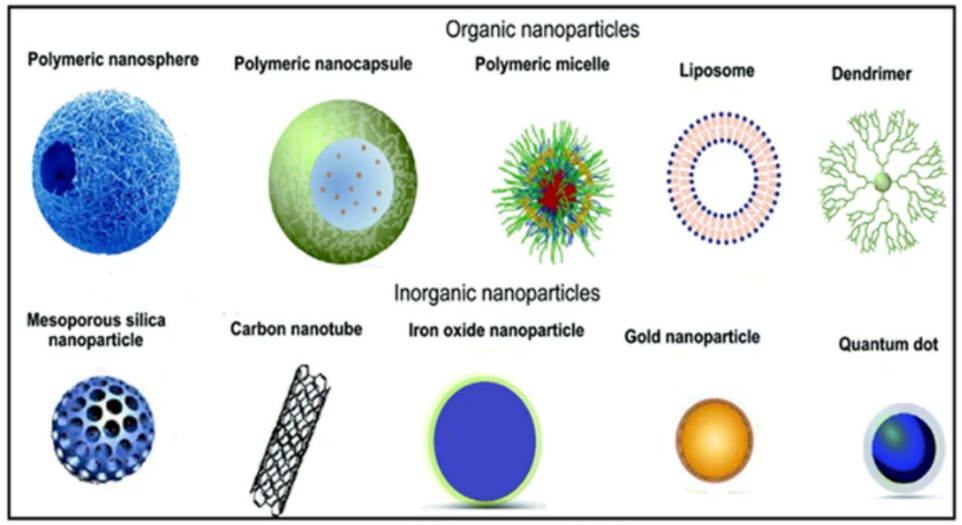
1. **Antibiotic resistance can arise both from mutations in the pre-existing genome of a bacterium and from the uptake of foreign DNA**
2. ***Antibiotic resistance is thought to have evolved long before naturally occurring antibiotics and their derivatives were used to treat human disease***
3. **ARGs (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)



Photodynamic therapy



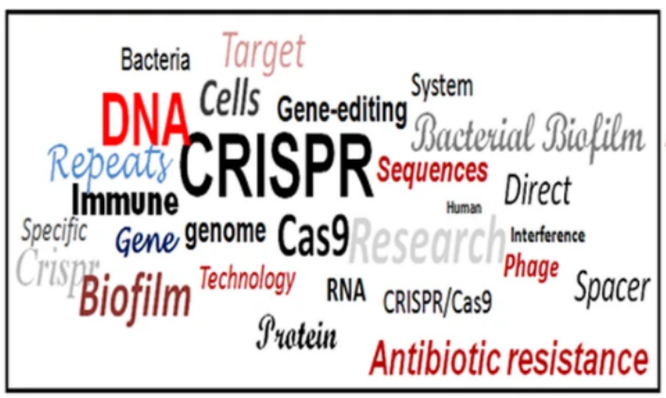
Nanoparticles

Inducing the dispersal of mature biofilms

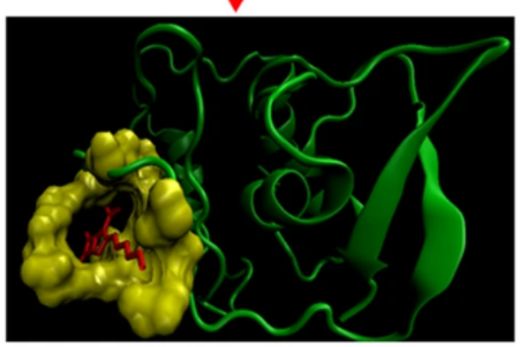
Natural compound

ALTERNATIVE THERAPY

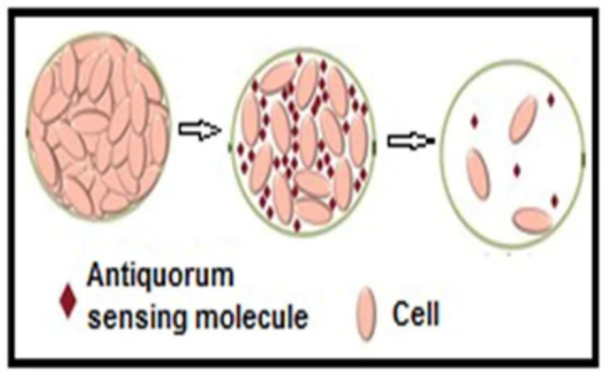
Phage therapy



CRISPR/Cas



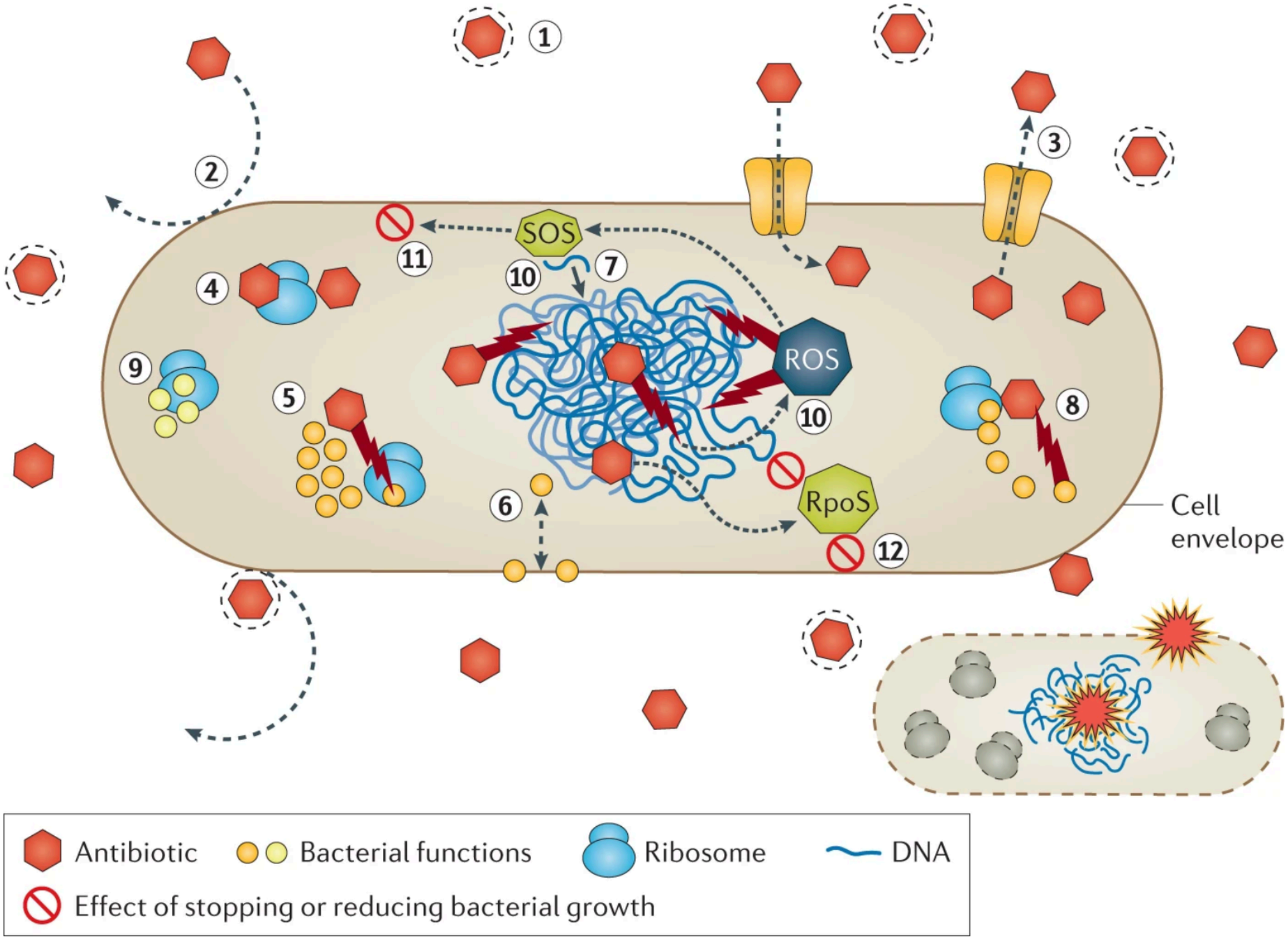
Matrix degrading enzyme



Anti-quorum sensing signalling molecules

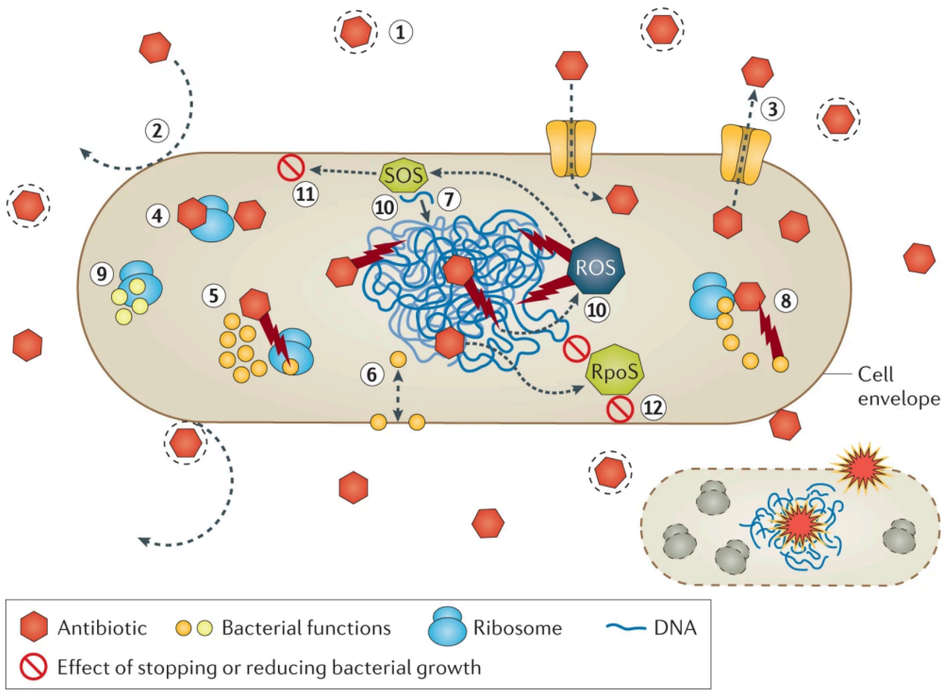
Sharma et al., 2019

Factors influencing the bactericidal activity of antibiotics in a susceptible bacterial cell



Baquero & Levin, 2020

Baquero & Levin, 2020



Only free drug is active (1) and protein binding, for example to albumin or other plasma proteins, can reduce the level of available and thus active drug, as commonly observed for β-lactams.

Uptake systems (such as porins) and barriers can prevent the drug from entering cells (2) or the drug is pumped out (3).

Greater or weaker target-binding affinity also influences activity (4).

The targeted function can also increase in the presence of the drug, thereby compensating for inhibition (5; for example, upregulation of RpoB by rifampin in mycobacteria).

The target function involves the build-up of a cellular structure with slow turnover (for example, peptidoglycan), which increases the amount of time for the antibiotic to kill (6).

The cells repair the damage produced by the drug (7), involving the SOS system.

The bacteria have inducible antibiotic-deactivating mechanisms (8; for example, β-lactamases).

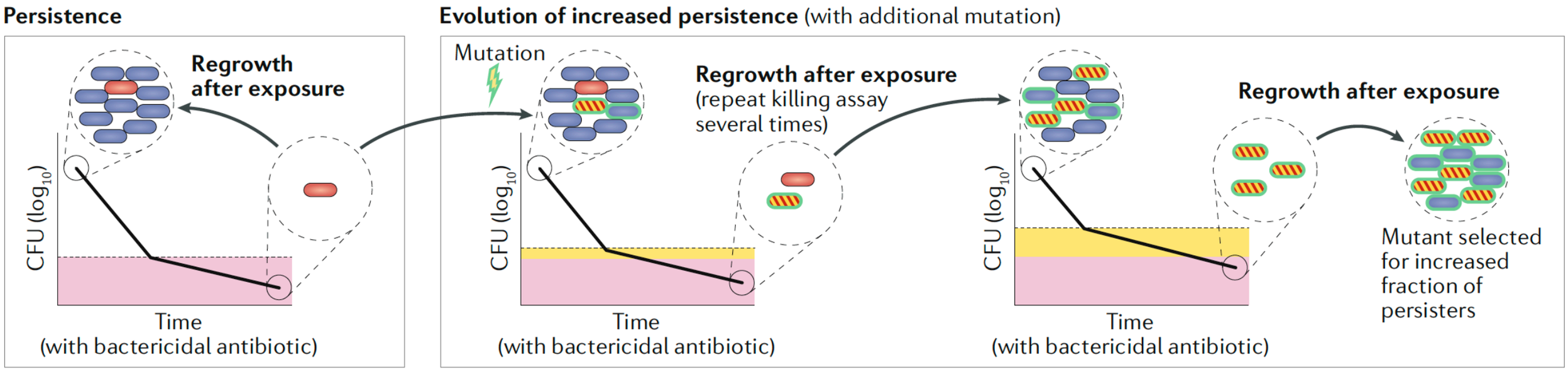
The bacteria use alternative functions, bypassing those that are inhibited (9).

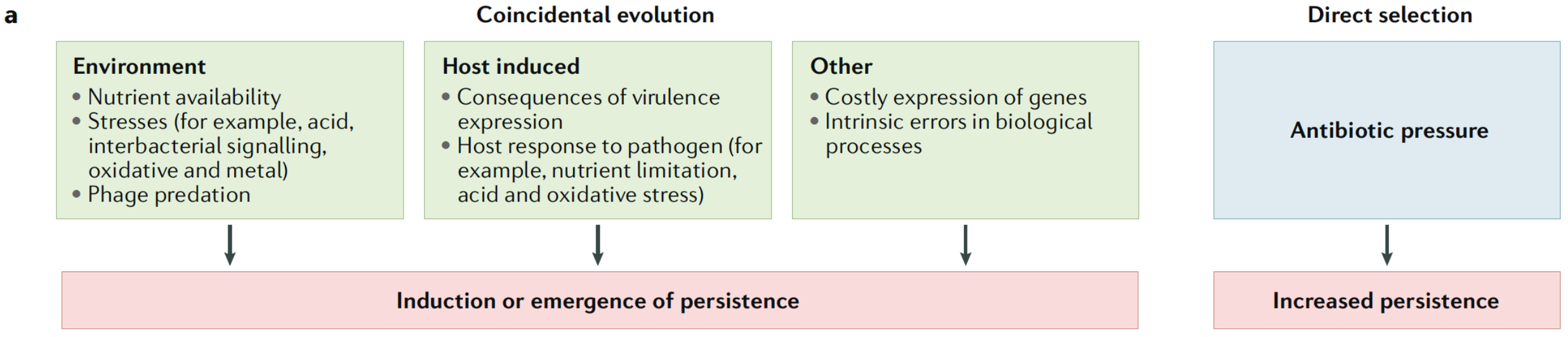
Antibiotics differ in the extent to which they induce reactive oxygen species (ROS; deleterious) or SOS (potentially protective) responses (10).

Low replication rates (involving the SOS system) reduce the killing activity (11).

Activation of the RpoS-mediated stringent response produces a kind of ‘stationary phase’, reducing bactericidal potency (12).
The two ultimate causes (bottom right) of a bactericidal effect are loss of spatial individuality by rupture of the limits with the environment (broken green line indicating disruption of the cell envelope) and loss of genetic individuality (broken blue line indicating disruption of the genome).

Persistence dynamics





Bakkeren et al., 2020

Antibiotic treatment failure is a substantial problem in modern medicine

Resistance, heteroresistance, tolerance and persistence

Resistance

The genetically encoded ability of cells to grow in the presence of an antibiotic. Resistance increases the minimum inhibitory concentration of an antibiotic compared with susceptible cells. The offspring remains resistant, even if grown in the absence of antibiotics.

Tolerance

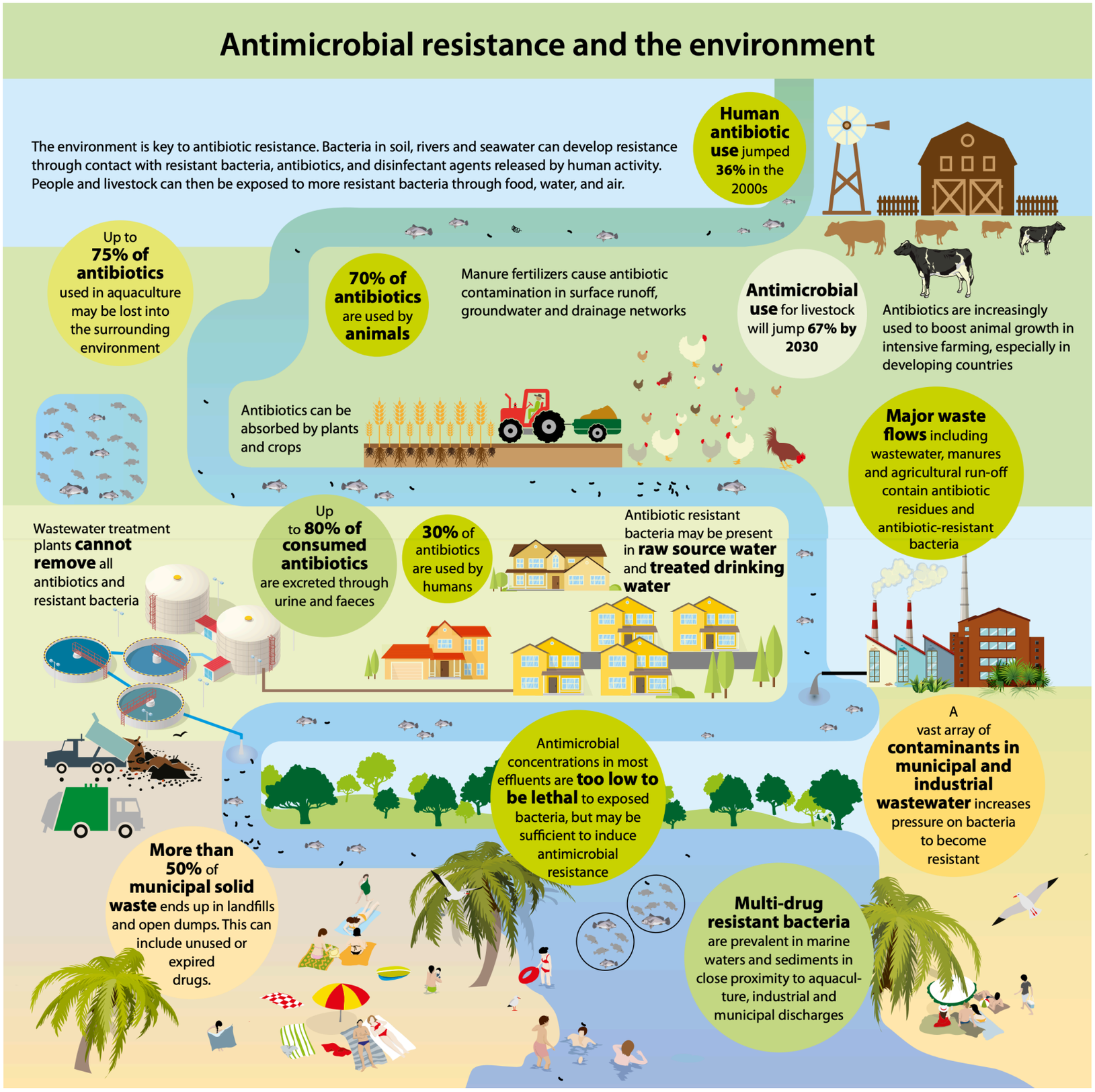
The ability of cells to survive in the presence of a bactericidal antibiotic to a higher extent than susceptible cells. This phenomenon pertains to all cells of the population and increases the minimum duration of killing in the presence of an antibiotic.

Persistence

The phenomenon that for a population in which two or more distinct subpopulations exist (susceptible and tolerant), treatment with a bactericidal antibiotic will kill the susceptible subpopulation quickly, simultaneous with a much slower killing of the tolerant subpopulation. This leads to biphasic killing curves characteristic of persistence. Persistence is not heritable (clones isolated from the tolerant subpopulation will again give rise to a mix of susceptible and tolerant cells). Persistence can also be called heterotolerance.

Bakkeren et al., 2020

Antimicrobial resistance global crisis



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

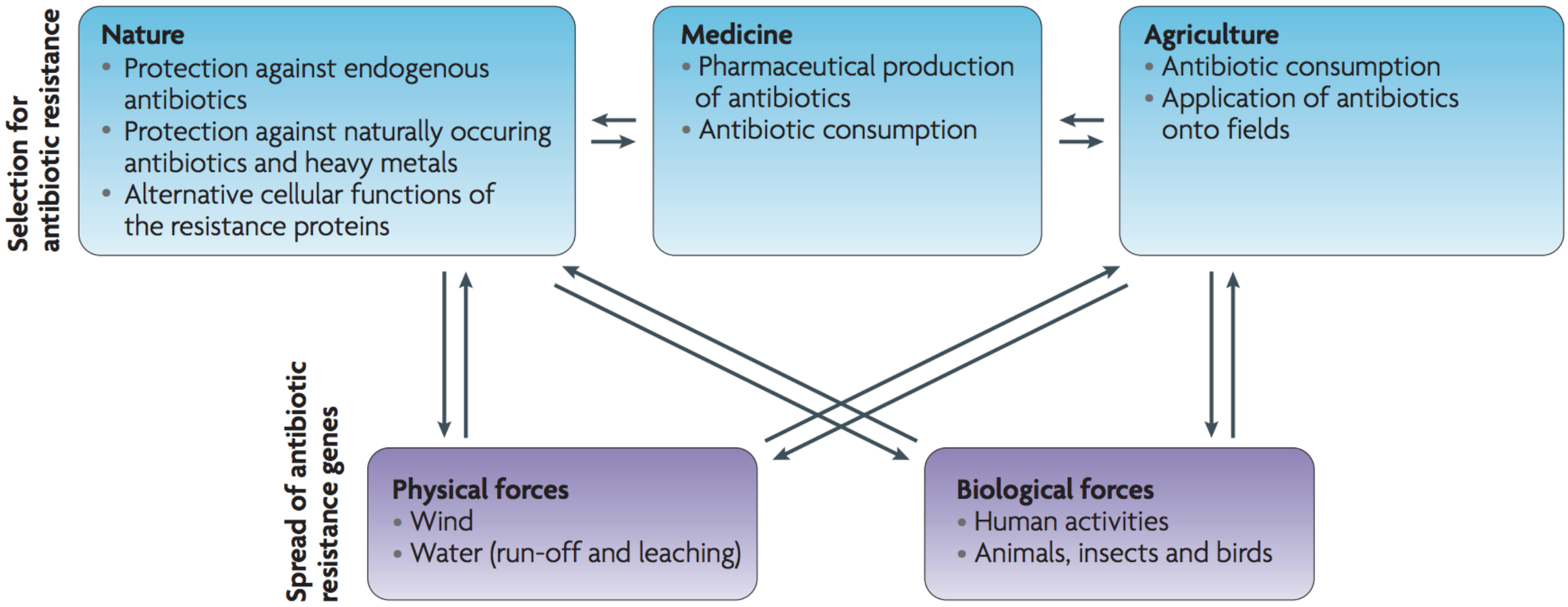
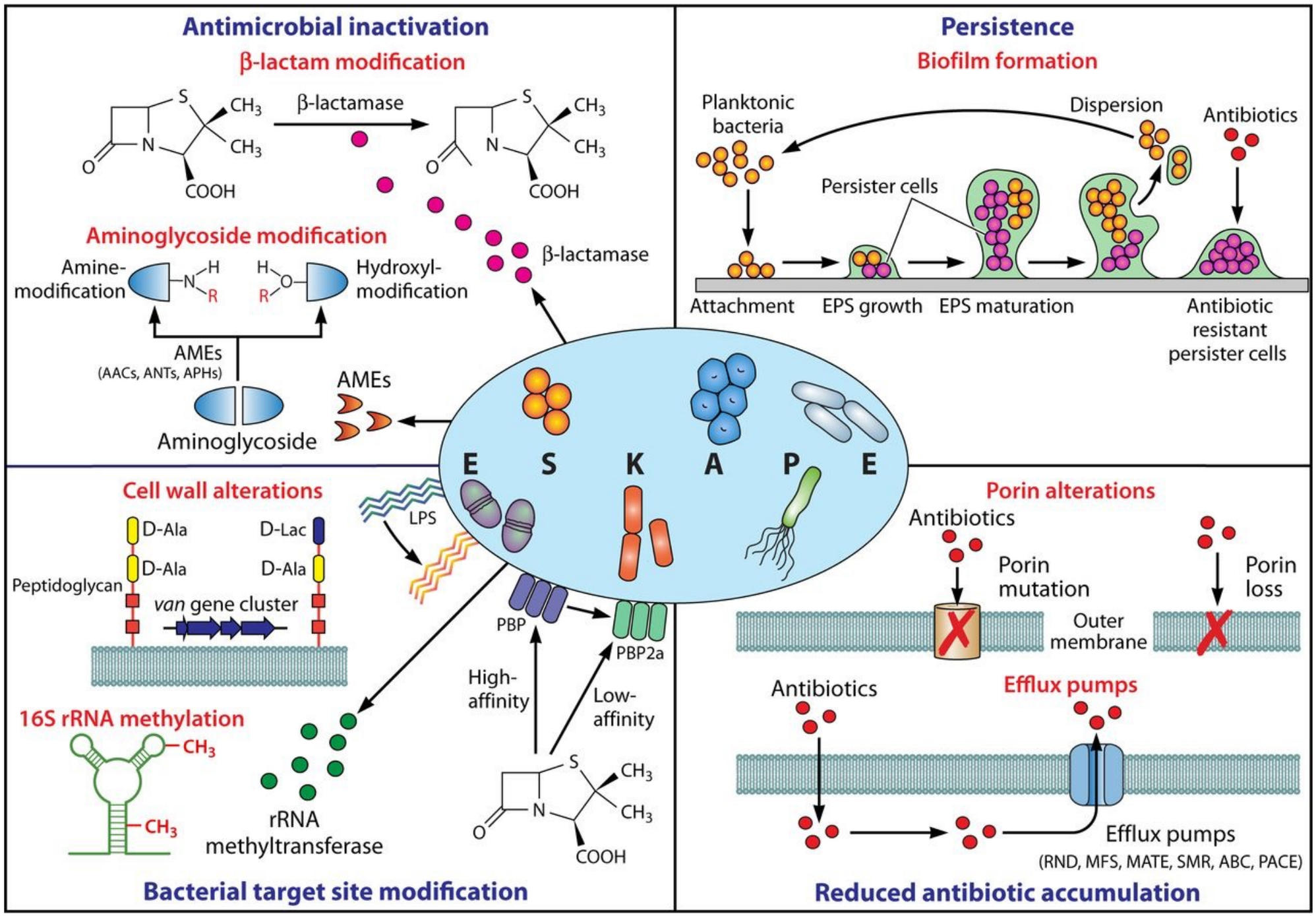


Figure 2 | **Sources and movement of antibiotic resistance genes in the environment.** Resistance genes exist naturally in the environment owing to a range of selective pressures in nature. Humans have applied additional selective pressure for antibiotic resistance genes because of the large quantities 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

Mediators of ESKAPE pathogen antimicrobial resistance

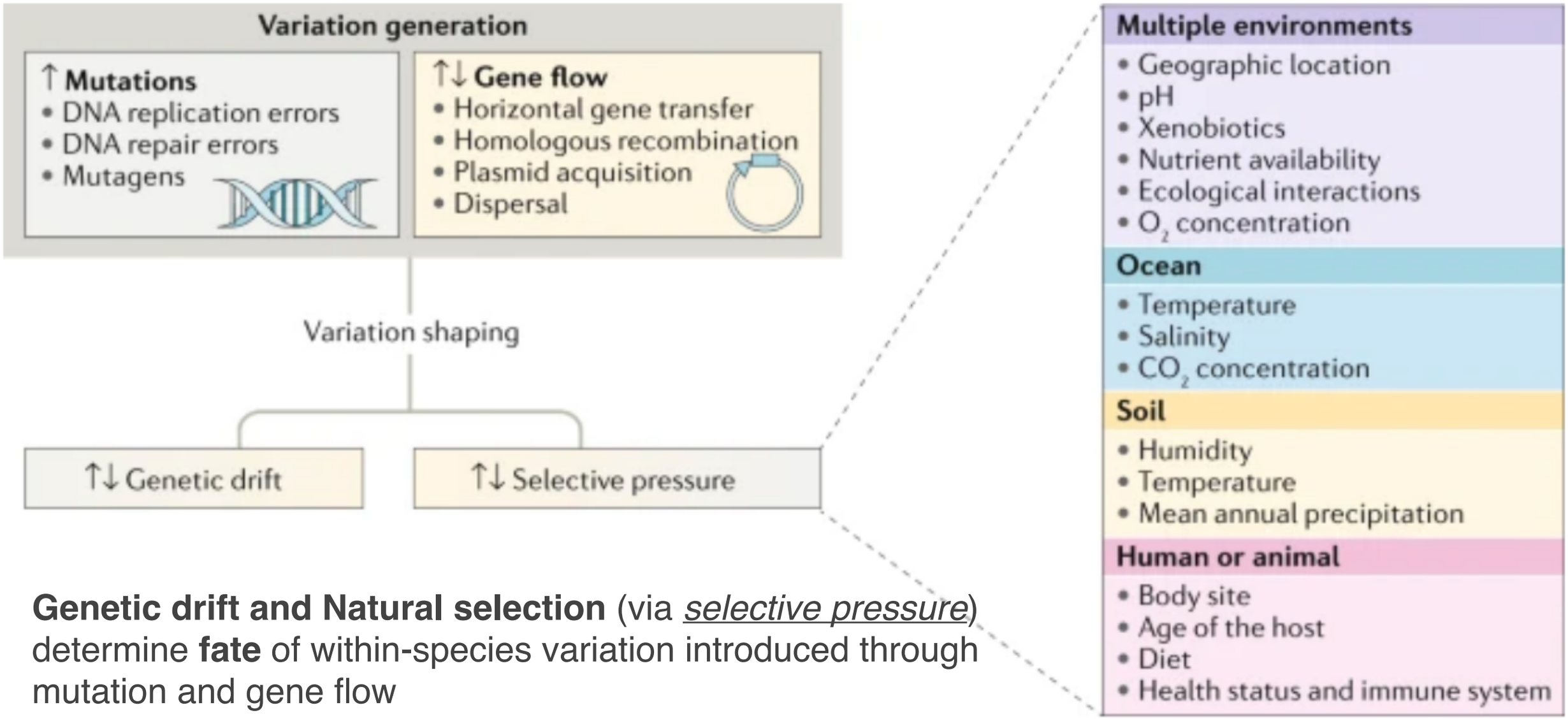
ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species)



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

To be or not to be?

Microbial species



Rossum et al., 2020

Genetic drift and Natural selection (via *selective pressure*) determine **fate** of within-species variation introduced through mutation and gene flow

Genetic drift randomly eliminates **genetic variations within a population**, whereas **natural selection maintains or eliminates** variations that respectively confer a **fitness** advantage or disadvantage

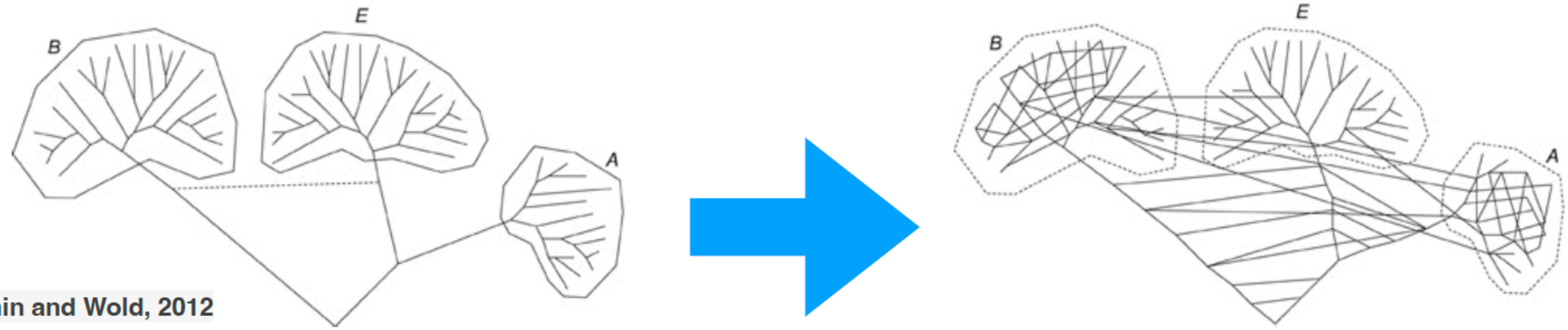
Effect of natural selection is limited by the background noise of genetic drift —> **Natural selection is driven by a multitude of biotic and abiotic factors** that *differentially influence the survival and replicative capability of species subpopulations*

Pangenome= core genome and accessory genome of a species (also cloud genome)

Bacterial genome is thought to consist of two distinct parts, the **core** genome and the **accessory** genome

The **core** genome comprises genes that are **essential** in most circumstances and might form the basis for Mayrian species that maintain **coherence** through homologous recombination.

The **accessory** genome encodes **special ecological adaptations** in genes that are readily gained and lost within the pangenome



Koonin and Wold, 2012

Bacterial gene-transfer processes, which are erratic and transfer only a small part of the genome

Not clear if there are more changes in core vs accessory genomes

Recombination is an important process driving the evolution of bacterial genomes **homologous and not-homologous kind** (i.e., **HGT**)

Mutations are the ultimate source of heritable variation for evolution

What is a microbial species?

specere, "to see".....something as an individual entity

15 **Mayr, E. in *The Species Problem* (ed. Mayr, E.) 1–22 (American Association for the Advancement of Science, Washington DC, 1957)**

A species as a group of interbreeding individuals that is isolated from other such groups **by barriers to recombination**.
If genetic exchange within a species is sufficiently extensive, and that between species is sufficiently low, species will be relatively homogeneous in themselves and ecologically distinct from other species

Cohan, F. M. What are bacterial species? *Annu. Rev. Microbiol.* 56, 457–487 (2002)

The ecological species concept, defines a species as a set of individuals that can be considered to be identical in all relevant ecological properties.
Bacteria have ecological species ('ecotypes') and occupy discrete niches and that periodic selection will purge genetic variation within each niche without preventing divergence between the inhabitants of different niches.

..using genomic DNA

Evolution

Evolutionary outcomes are determined by core factors that shape the diversity of life: **adaptation, chance and history.**

Adaptation reflects the power of natural selection to drive populations along evolutionary paths to phenotypes of high fitness. If few paths are available, replicate populations will follow repeatable, perhaps even predictable, outcomes.

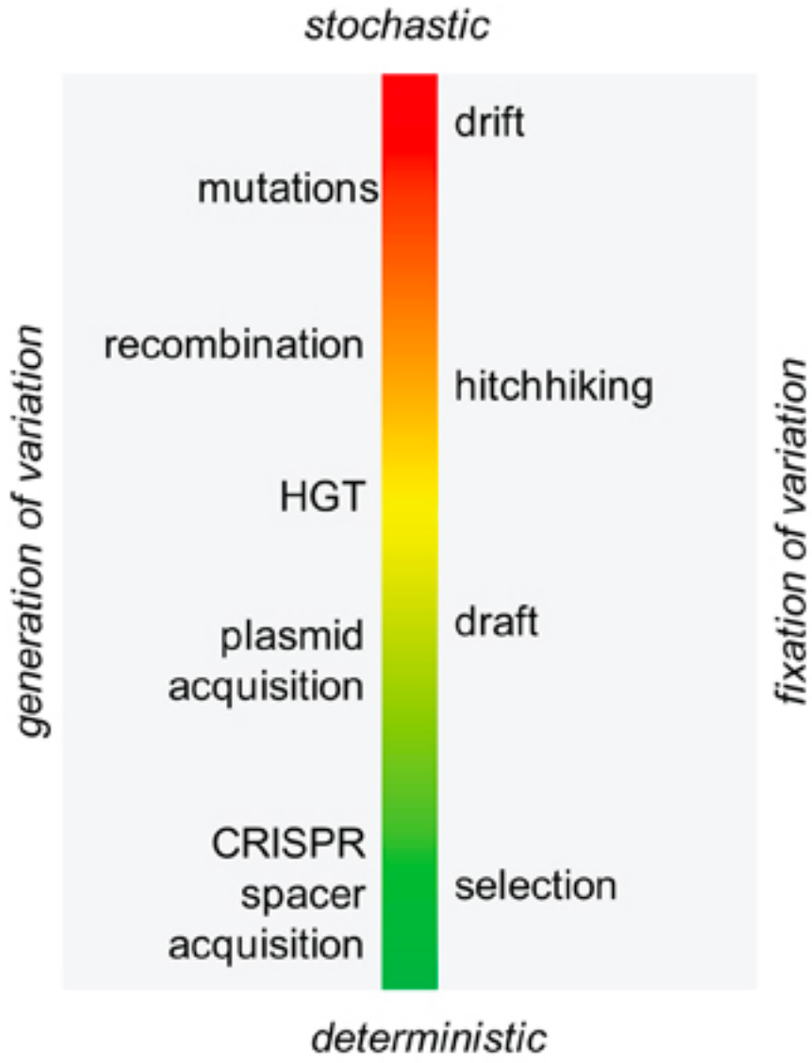
By contrast, chance and history promote evolutionary divergence.

Chance causes divergence between populations through stochastic differences in the occurrence and success of newly arising mutations.

History, defined here as differences in the genetic starting points of selected populations, promotes divergence if evolutionary opportunities or constraints are contingent on specific genotypes.

Determining the relative contribution of these forces, and how this might depend on the selective environment, is crucial to the goal of predicting evolutionary outcomes.

Evolution can be more adequately depicted as a **continuum of processes** from completely random ones, under the Wrightean modality defined by random variation and random fixation of changes via genetic drift; to the Darwinian modality with random changes fixed by the deterministic process of selection; to the Lamarckian mode in which both variation and fixation are deterministic.



Smith Chelsea E., Smith Adam N. H., Cooper Tim F. and Moore Francisco B.-G. 2022

Eugene V. Koonin* and Yuri I. Wolf, 2015

Disentangling the effects of selection and loss bias on gene dynamics

Iranzo et al., 2017

The **evolution of microbial genomes** is generally interpreted in terms of the interplay between three factors:

★ (i) **gene gain**, via horizontal gene transfer (HGT) and gene duplication;

★(ii) **gene loss**, via deletion;

★(iii) **natural selection** that affects gene fixation and maintenance

The intrinsic bias toward DNA deletion (and hence gene loss) that characterizes mutational processes in prokaryotes results in nonadaptive genome reduction, whereas selection contributes to maintaining slightly beneficial genes

Mutations, I

- Mutation is **heritable change** in genome **base sequence** (mother —> cell progeny)
- Mutations —> changes cell properties
- Mutations are beneficial, some are detrimental, but most are **neutral** w. **no effect**
- **Spontaneous mutations** occur without external intervention (occasional errors in pairing of bases by DNA polymerase during replication)
- **Induced mutations,** caused by agents in environment and by humans
- Exposure to natural radiation that **alters structure of bases** in DNA, or from a variety of chemicals that **chemically modify** DNA

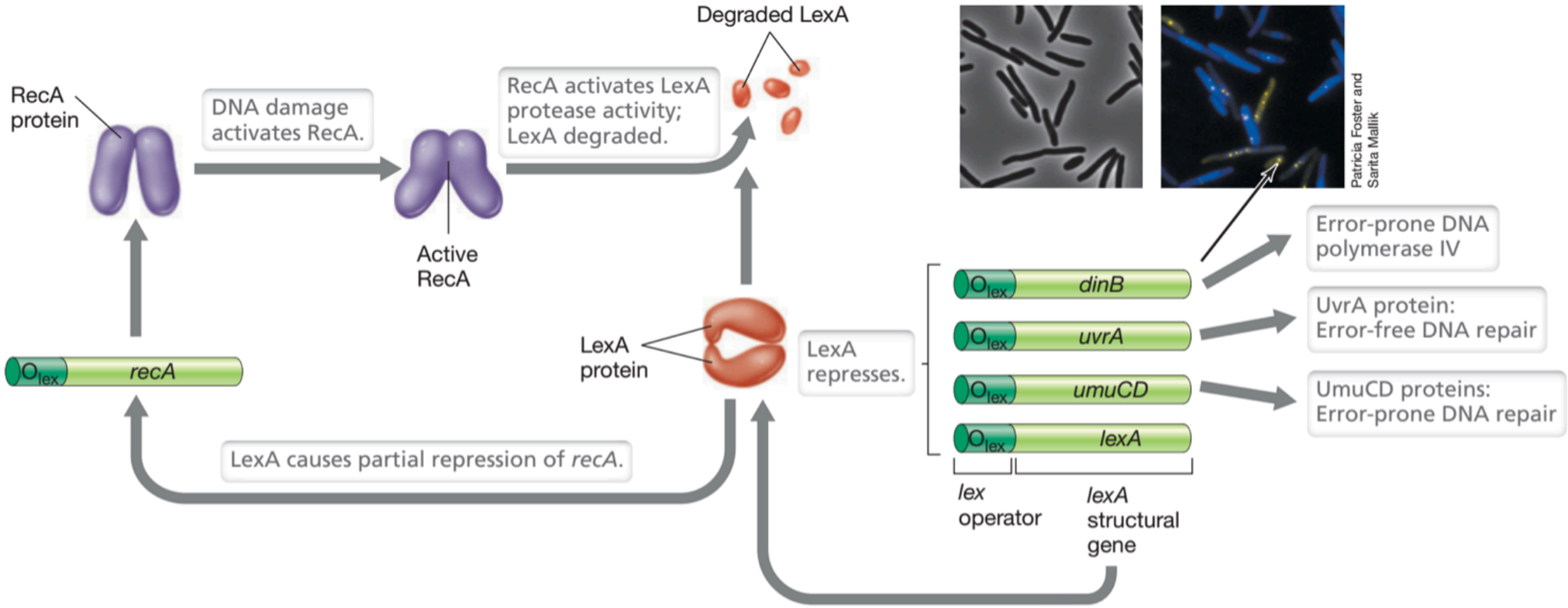
TABLE 11.2 Chemical and physical mutagens and their modes of action

Agent	*Action*	*Result*
Base analogs		
5-Bromouracil	Incorporated like T; occasional faulty pairing with G	AT → GC and occasionally GC → AT
2-Aminopurine	Incorporated like A; faulty pairing with C	AT → GC and occasionally GC → AT
Chemicals reacting with DNA		
Nitrous acid (HNO_2)	Deaminates A and C	AT → GC and GC → AT
Hydroxylamine (NH_2OH)	Reacts with C	GC → AT
Alkylating agents		
Monofunctional (for example, ethyl methanesulfonate)	Puts methyl on G; faulty pairing with T	GC → AT
Bifunctional (for example, mitomycin, nitrogen mustards, nitrosoguanidine)	Cross-links DNA strands; faulty region excised by DNase	Both point mutations and deletions
Intercalating agents		
Acridines, ethidium bromide	Inserts between two base pairs	Microinsertions and microdeletions
Radiation		
Ultraviolet (UV)	Pyrimidine dimer formation	Repair may lead to error or deletion
Ionizing radiation (for example, X-rays)	Free-radical attack on DNA, breaking chain	Repair may lead to error or deletion

Madigan et al. 2020

DNA Repair & SOS System, I

- DNA damage (e.g. large-scale damage from highly mutagenic chemicals or large doses of radiation), may cause lesions that **interfere with replication**
- **DNA replication will stall and lethal breaks in the chromosome** —> activate the SOS repair system
- **SOS system DNA is repaired without a template** —> with **random incorporation of dNTPs (TRANSLESION) —> can cause mutations**
- Mutations may be less detrimental to cell survival than chromosome breaks
- In *E. coli* the **SOS repair** system forms a regulon, controls the transcription of approximately 40 genes —> **for DNA damage tolerance and DNA repair**



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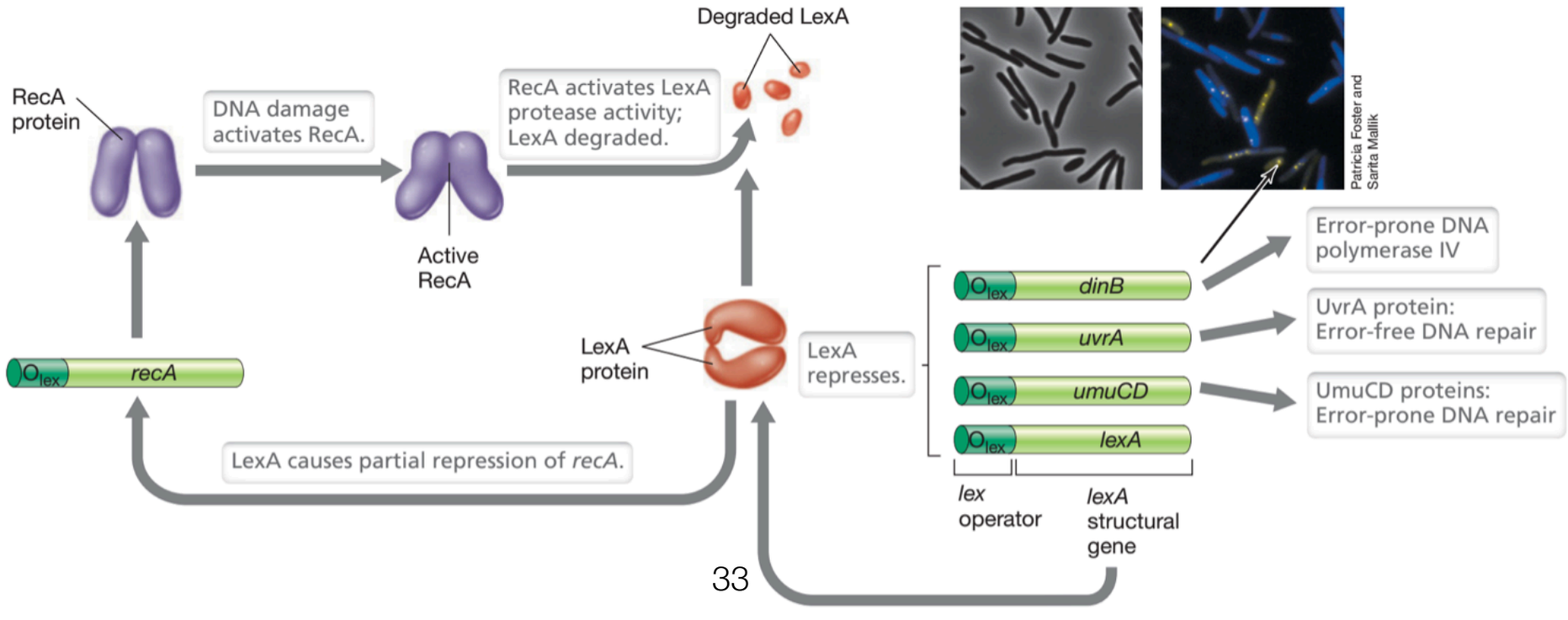
DNA Repair & SOS System, II

- In DNA damage tolerance, DNA lesions remain in the DNA, are bypassed by specialized DNA polymerases that can move past DNA damage—> translesion synthesis
- In *E. coli*, **2 error-prone repair polymerases are DNA polymerase V** (encoded by umuCD genes), **DNA polymerase IV** (by dinB gene)—> many mutations
- **LexA is a repressor** that normally prevents SOS expression
- **RecA protein**, which normally functions in **genetic recombination is activated by DNA damage (ssDNA when replication stalls)**
- Activated **RecA stimulates LexA to inactivate itself by self-cleavage**—> coordinated expression of proteins that participate in DNA repair
- Once original DNA damage has been repaired, the SOS regulon is repressed



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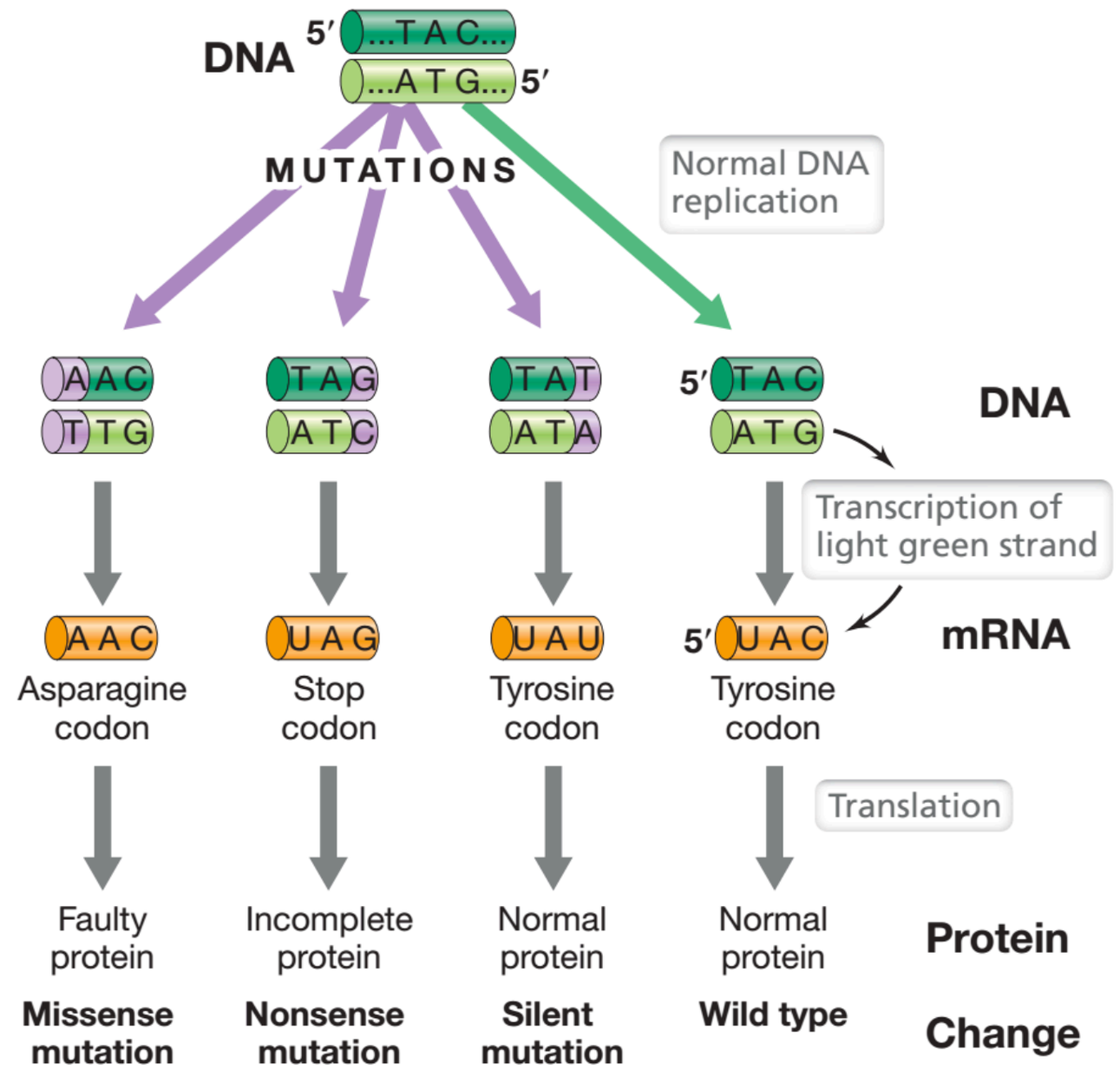
Mutations, II

- One base pair mutations are **point mutations** and occur when a **single base-pair substitution** takes place in DNA
- Results from a point mutation depends on exactly **where in genome** the mutation occurs and **the nature of the nucleotide change**



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Mutations, III

- **Spontaneous mutation rate** is low, speed at which many prokaryotic cells divide and their characteristic exponential growth —> **mutations accumulate in a population surprisingly fast**
- Single mutation brings about only a small change in cell
- **Genetic exchange often generates much larger change**
- ***Mutation + genetic exchange fuel the evolutionary process***
- If damaged DNA can be corrected before cell division, no mutation will occur

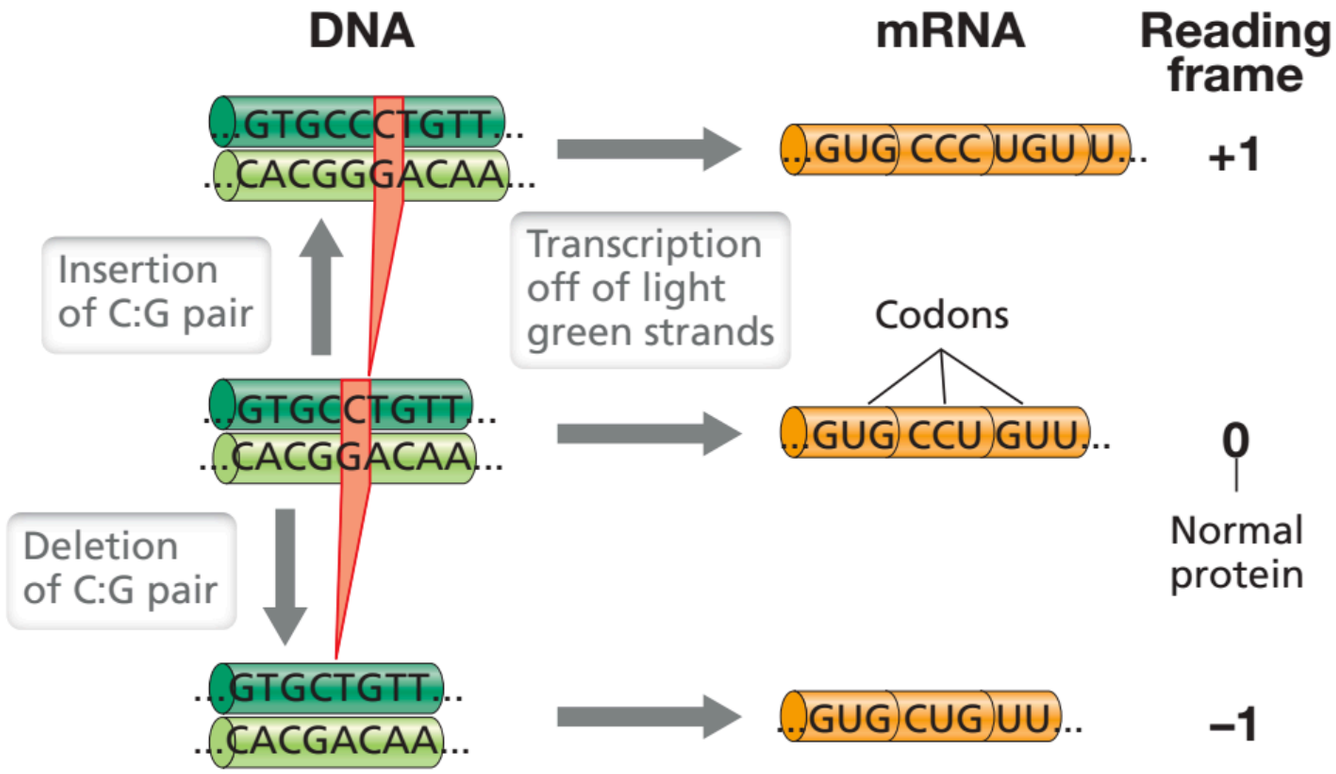
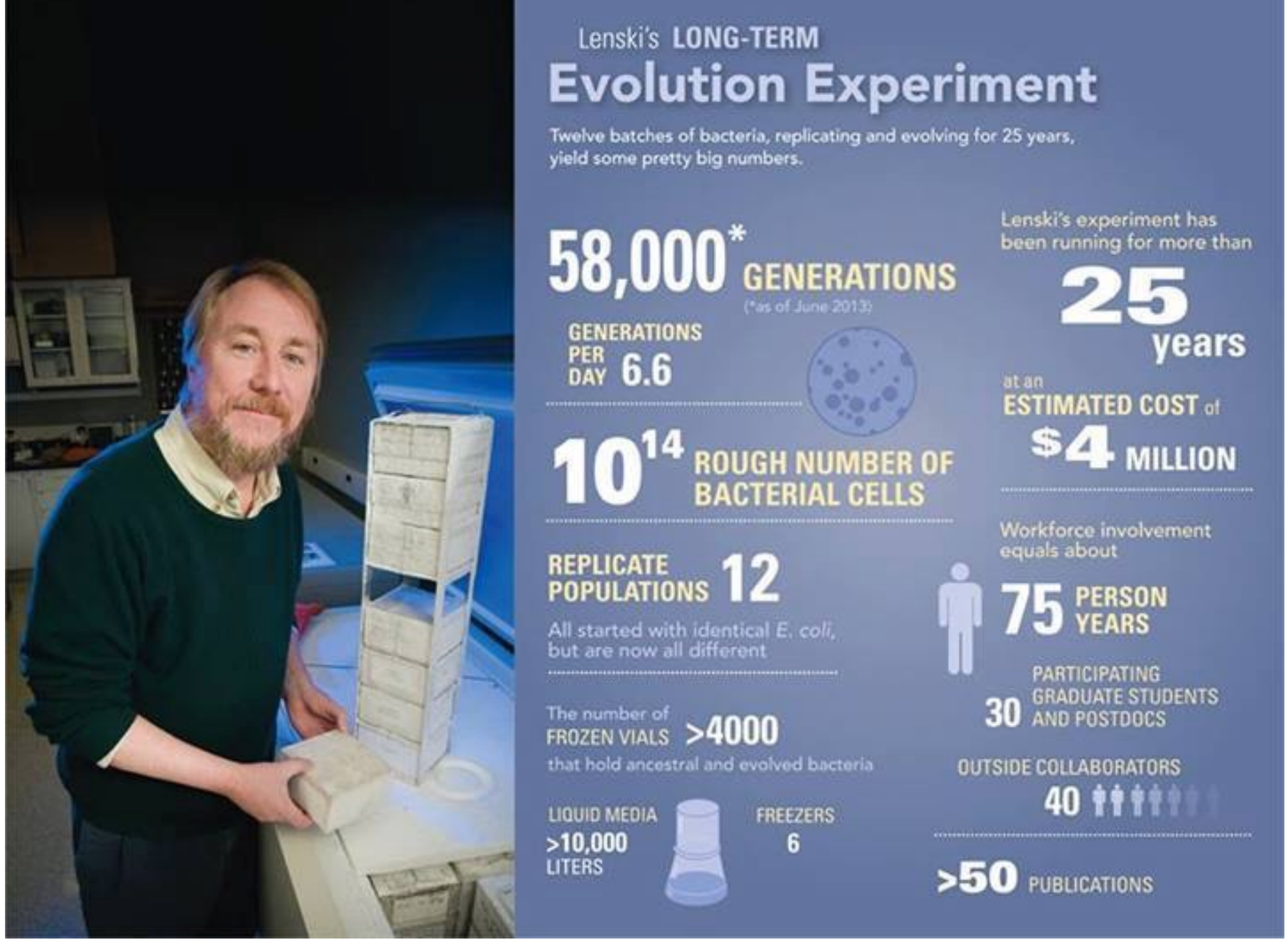


Figure 11.5 Shifts in the reading frame of mRNA caused by insertions or deletions. The reading frame in mRNA is established by the ribosome, which begins at the 5′ end (toward the left in the figure) and proceeds by units of three bases (codons). The normal reading frame is referred to as the 0 frame, that missing a base the −1 frame, and that with an extra base the +1 frame.

Madigan et al. 2020

Microbial species & Mutations

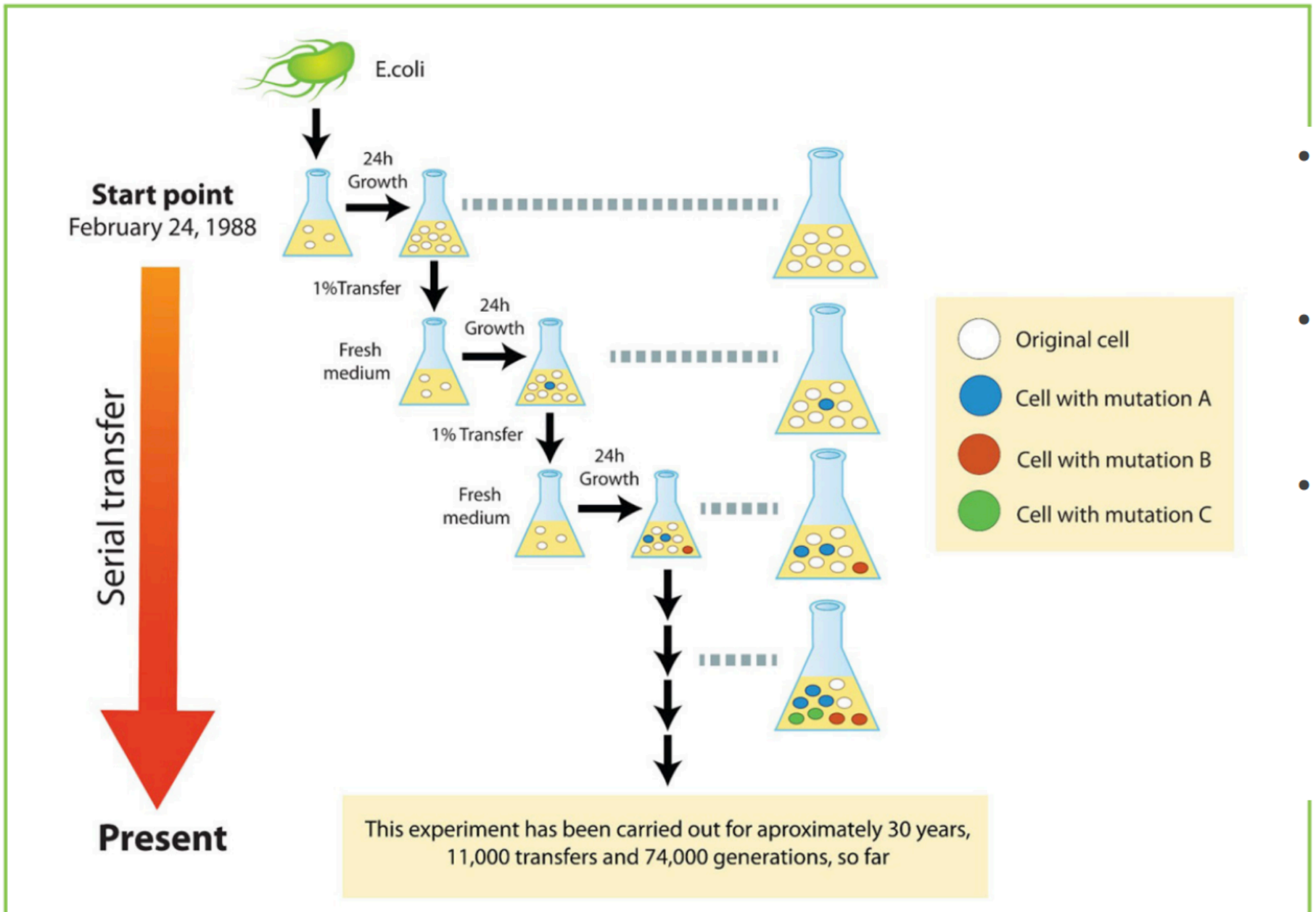
- The **rate of accumulation of mutations** within a lineage of bacteria depends on the **mutation rate** as well as on **natural selection and genetic drift**, which act upon the mutations
- **Non-lethal rates** of mutation from 10^{-9} to 10^{-3} mutations per genome per generation (in Vibrio species)
- **Not all portions** of bacterial **genome** are equally subject to mutations
- Mutation accumulation rates are **higher in accessory genes** than in core genes, unless a core gene is located near accessory genes or mobile genetic elements, and higher in secondary chromosomes than in primary chromosomes
- **Deletions are more frequent than insertions**, and non-functional sequences are readily lost from bacterial genomes
- Mutations that arise in one genome can be **passed vertically to descendants or horizontally to neighbouring cells**



Much of our understanding of microbial evolution and adaptation has come from experimental evolution [44], and reviews of the topic can be found elsewhere [45]. One example of the power of evolutionary experiments is the Long-Term Evolutionary Experiment (LTEE), started by Richard Lenski 30 years ago [46], which documented adaptive bacterial evolution in constant laboratory conditions and showed that different mutation rates can emerge in previously identical populations. This LTEE has also demonstrated the evolution of new metabolic capabilities [47] and the emergence of ecological interactions between two interdependent subpopulations harbouring different mutations [48,49]. Experimental evolution has

Long-Term-Evolutionary Experiment (LTEE), Richard Lenski

12 populations of *Escherichia coli* in a simple laboratory environment (medium) for >30 years and 75000 generations from February 1988



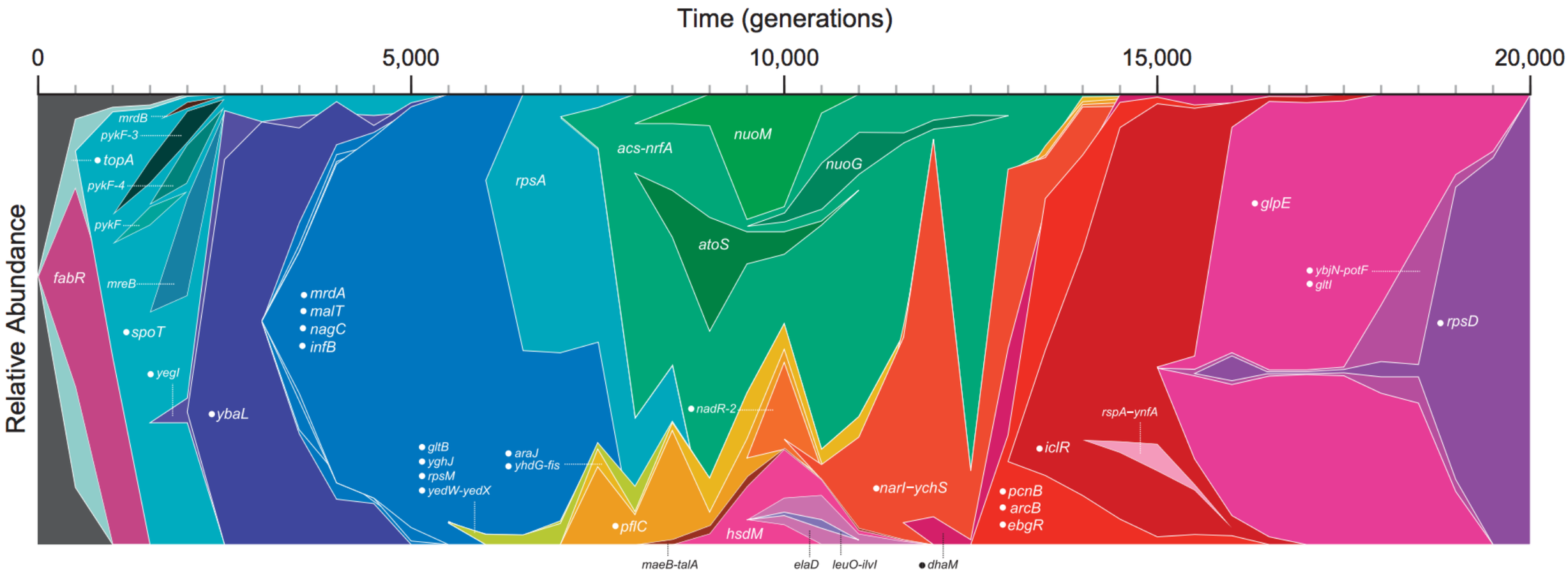
Aguilar, 2019

Figure 3

- 12 replicate populations have been propagated in a glucose-limited medium
- Daily 100-fold dilutions and regrowth allow ~6.6 generations per day
- Every 500 generations (75 days), after the transfers into fresh medium, glycerol was added as a cryoprotectant to the remaining cultures, which were then stored for later research at –80°

12 populations of Escherichia coli in a simple laboratory environment (medium) for >25 years and 60 000 generations from February 1988

Richard Lenski



In population Ara–1, two lineages coexisted from ~ 7000 to ~ 14 000 generations, before one drove the other extinct. Muller plot showing the relative abundances of 42 mutations found in population Ara–1 during its first 20 000 generations

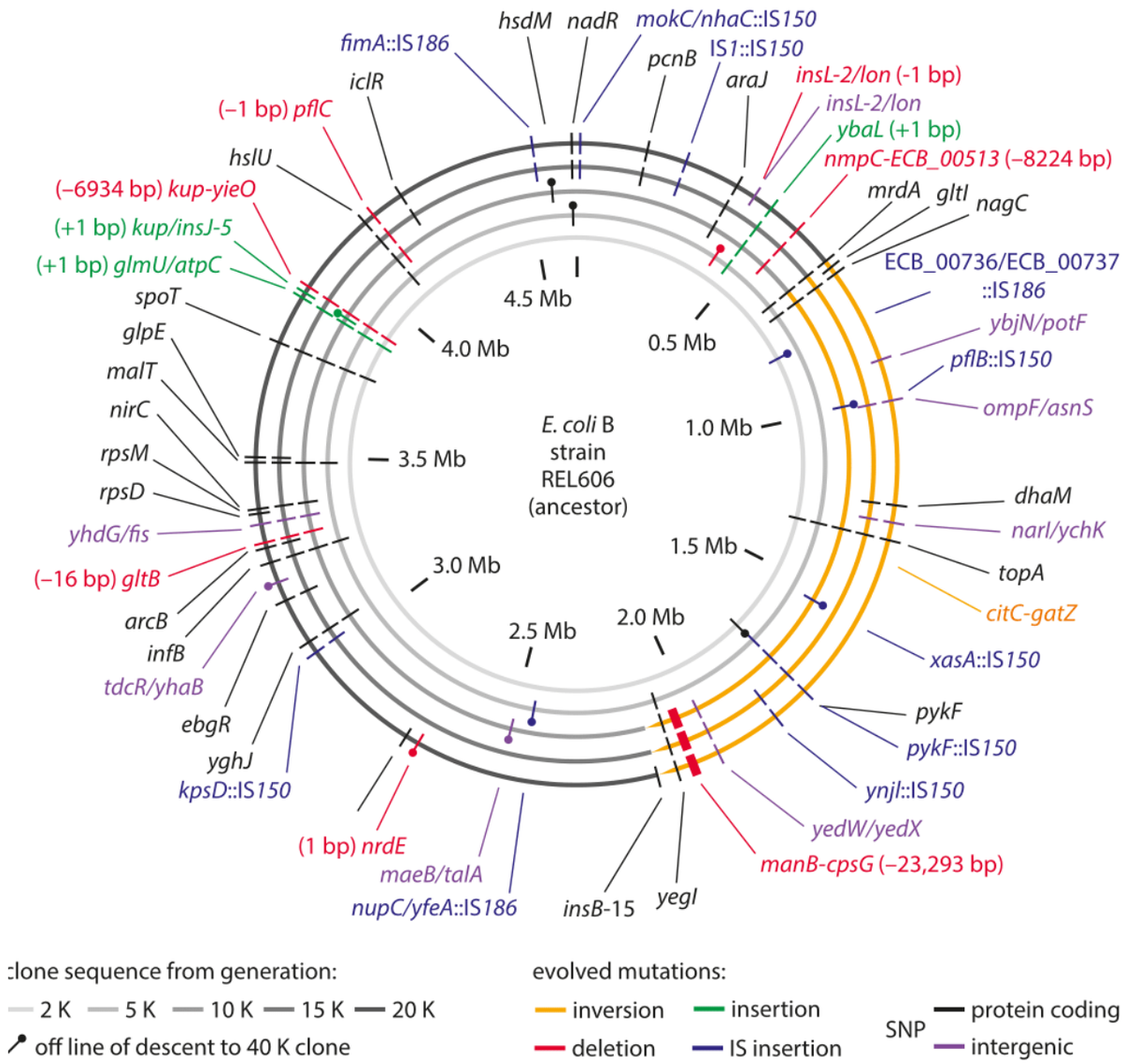
Sequencing measurements of fixed mutations over 20,000 generations in *E. coli*

Long-term experiment: compare full genome sequence at different times to reference sequence for the genome at the time the experiment started

Labels in outer ring show the specific mutations that were present after 20,000 generations

Sequencing of 19 whole genomes detected **25 synonymous mutation** (indicating neutral rather than selective changes) that got fixed in the 40,000 generations of experiment

Inference mutation rate is about 10^{-10} mutations per bp per replication in measured conditions



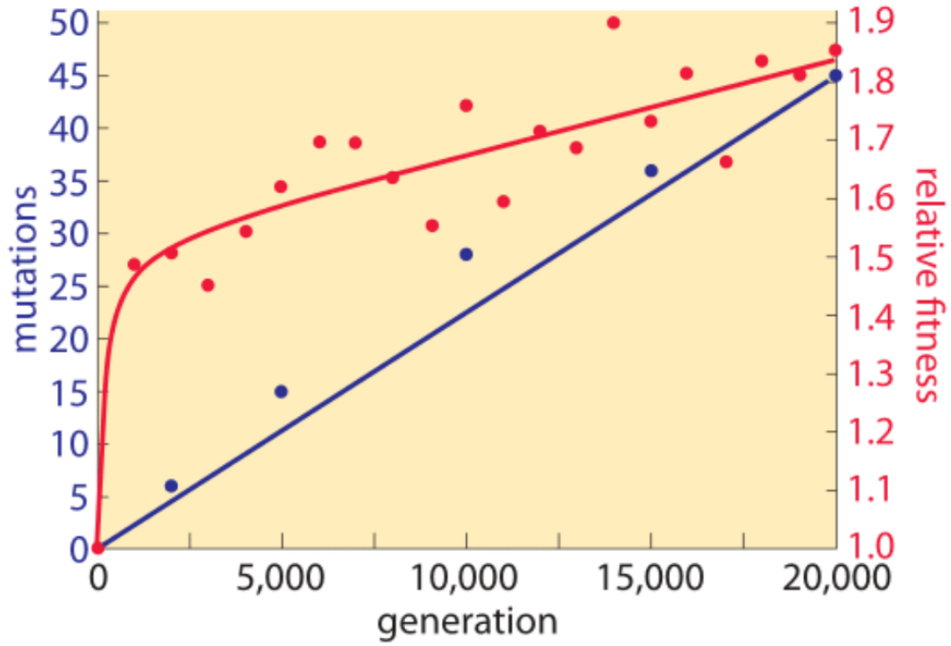


Figure 2: Mutation accumulation and fitness over time. Sequencing measurements make it possible to examine the rate of mutation accumulation and the corresponding fitness over time. Adapted from J. E. Barrick et al. Nature, 461:1243, 2009.

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.

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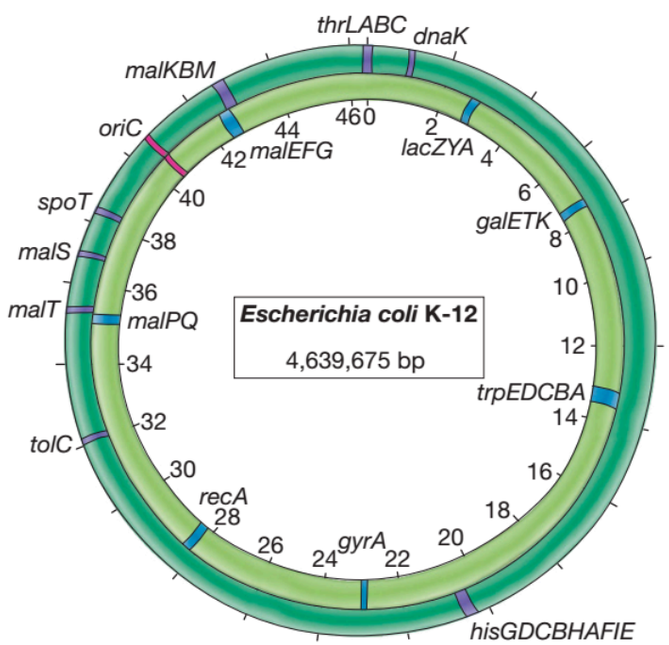
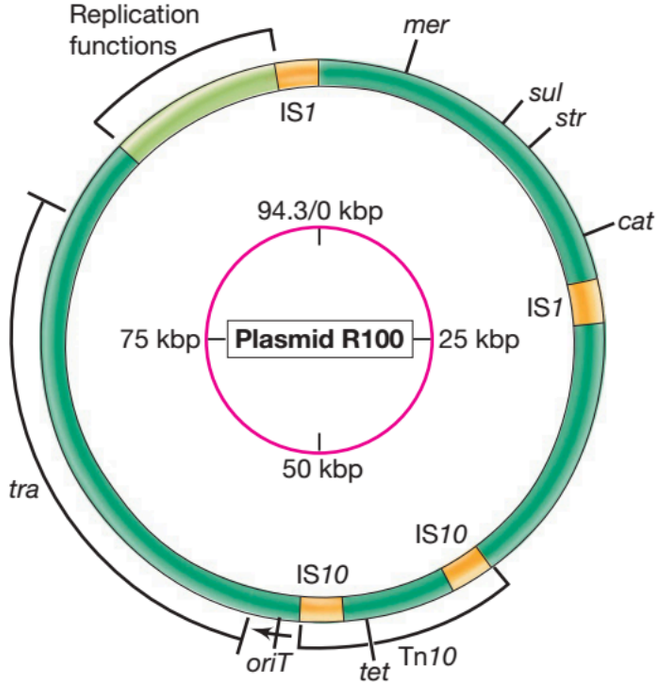


E. coli strain K-12



Resistance plasmid R100

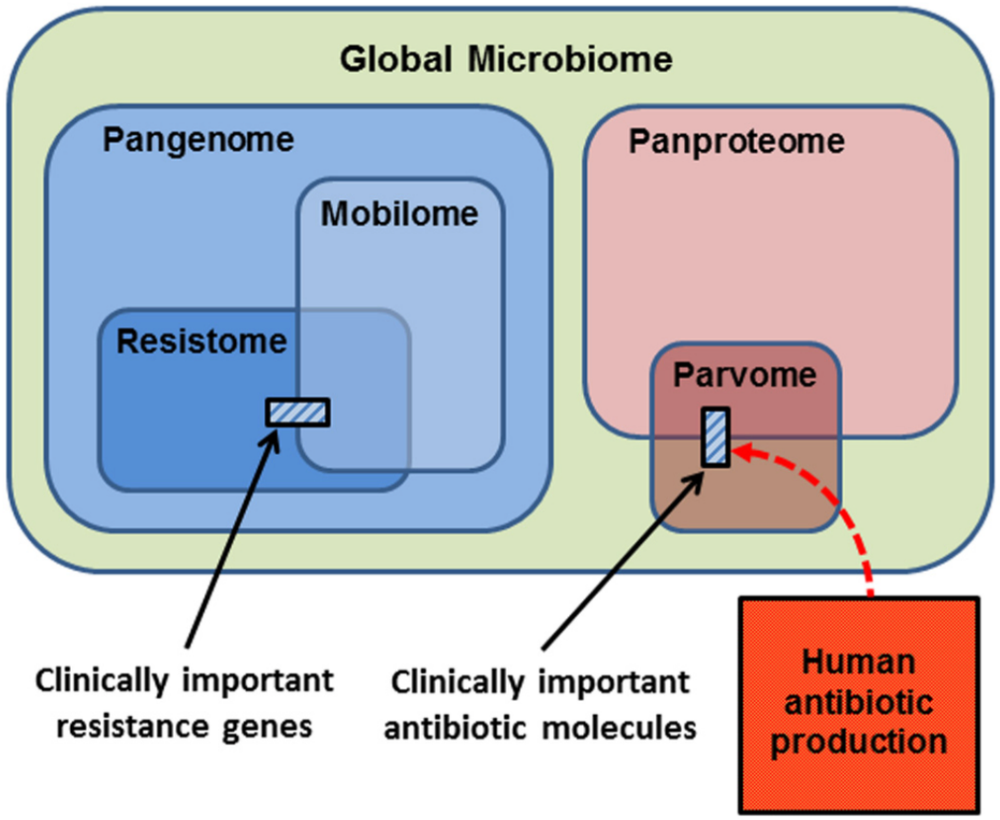
Madigan et al. 2020

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 via horizontal gene transfer (HGT) and* **contribute to evolution (by shuffling genes)**

- **Plasmids**
- **Bacteriophages**
- **Insertion sequences (IS) and transposons (Tn)**
- **GEnomic (PAthogenicity islands, PAIs) island (GEI)**

Chinese box: connectivity among pangenome, mobilome, resitstome



Gillings, 2013

- **Parvome:** world of small bioactive molecules
- **Resistome**: world of potential resistance determinants
- The resistome comprises the genes that potentially encode resistance to antibiotics.
- **The mobilome comprises the mobile proportion of bacterial genomes. The mobilome and resistome overlap, since many resistance genes are located on mobile elements**.
- Both the resistome and mobilome are a subset of the total coding capacity of prokaryotic cells, the **pangenome**, which is expressed as the panproteome

Mobile Genetic Elements, II

- 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

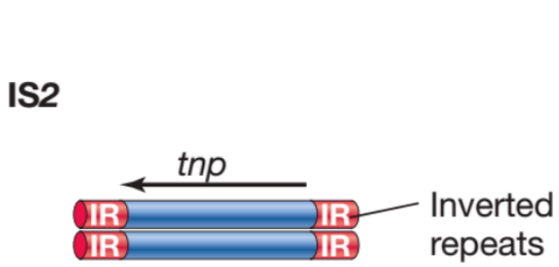
C. **Virulent genes, antibiotic and metal resistance**, other special metabolism

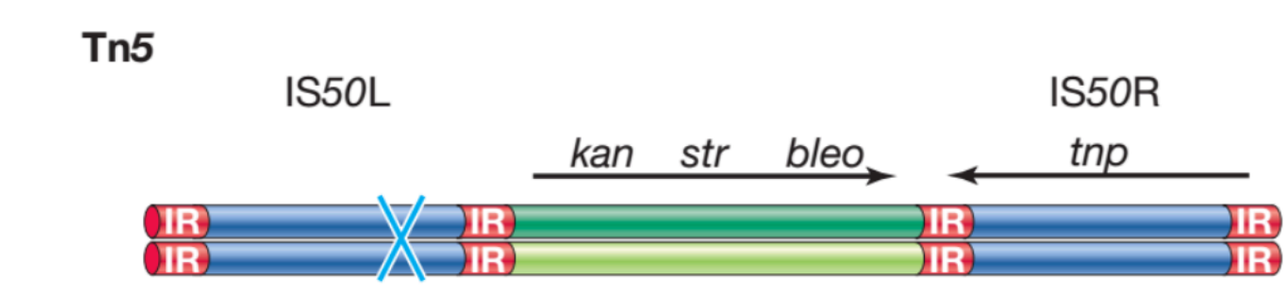
Mobile Genetic Elements, III

- **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)**

★ **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

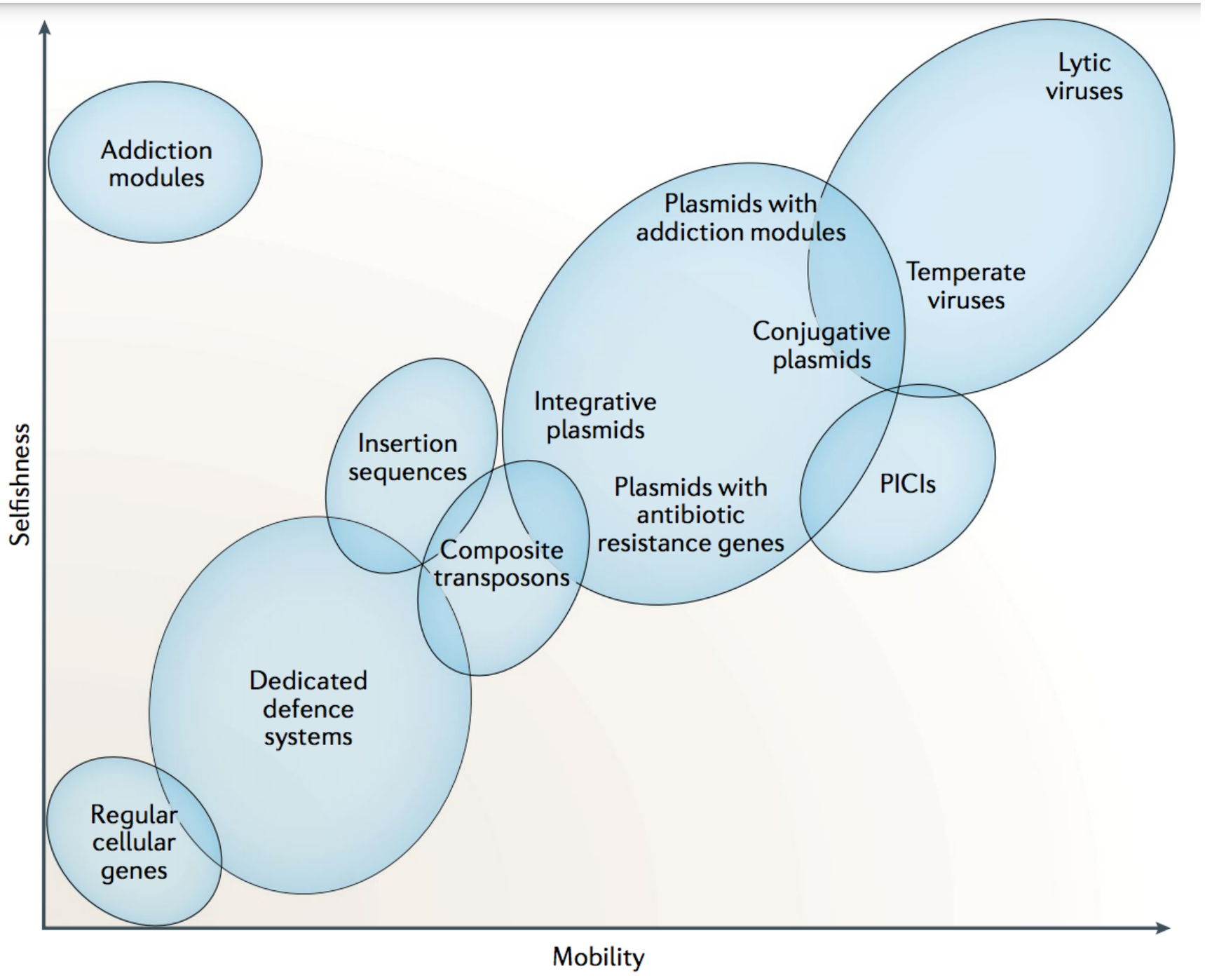
Mobile Genetic Elements, IV

- **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

Mobilome importance

- Understanding evolution driven by HGT—> positive/negative/neutral
    - **Only genes involved in key informational and metabolic pathways are subject to strong selection**
- Insight into microbial behavior and its dynamic change due to mobilome
- MGEs can confer **profoundly different phenotypes** on different strains of the same species
- Human activities and behaviors provide **selective pressures** that shape mobile gene pools, and that acquisition of mobile genes is important for colonizing specific human populations (**gut microbiome**)
- Focus on human health: microbial virulence and microbial defense

Persistence strategies of MGEs



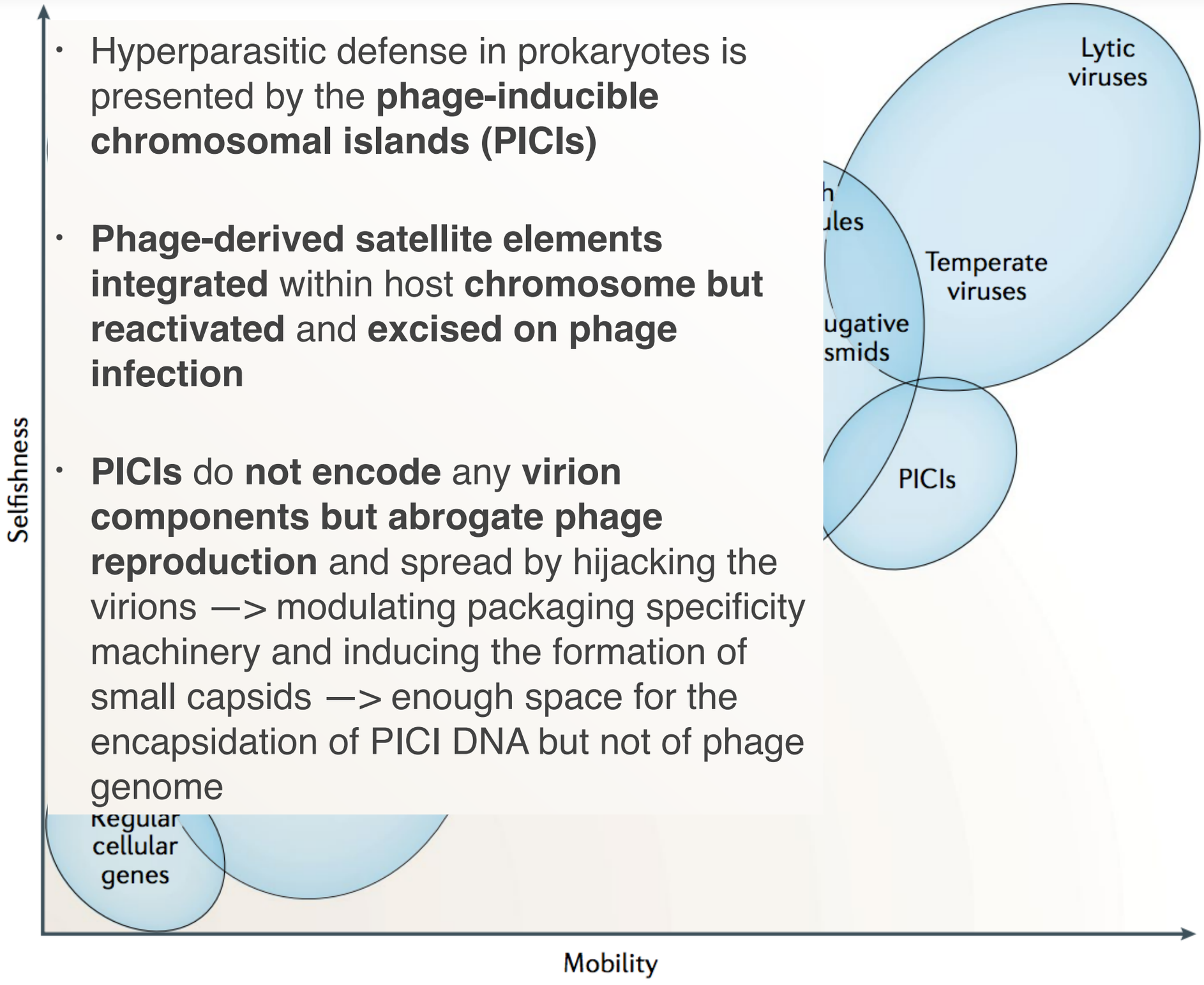
Koonin et al., 2020

Fig. 1 | **Distribution of mobile genetic elements and defence systems in the virtual space bounded by the axes of selfishness and mobility.** The plot is a conceptual diagram, and the positions of each class of elements are approximate. PICI, phage-inducible chromosomal island.

- Hyperparasitic defense in prokaryotes is presented by the **phage-inducible chromosomal islands (PICIs)**

- **Phage-derived satellite elements integrated** within host **chromosome but reactivated** and **excised on phage infection**

- **PICIs** do **not encode** any **virion components but abrogate phage reproduction** and spread by hijacking the virions —> modulating packaging specificity machinery and inducing the formation of small capsids —> enough space for the encapsidation of PICI DNA but not of phage genome



Koonin et al., 2020

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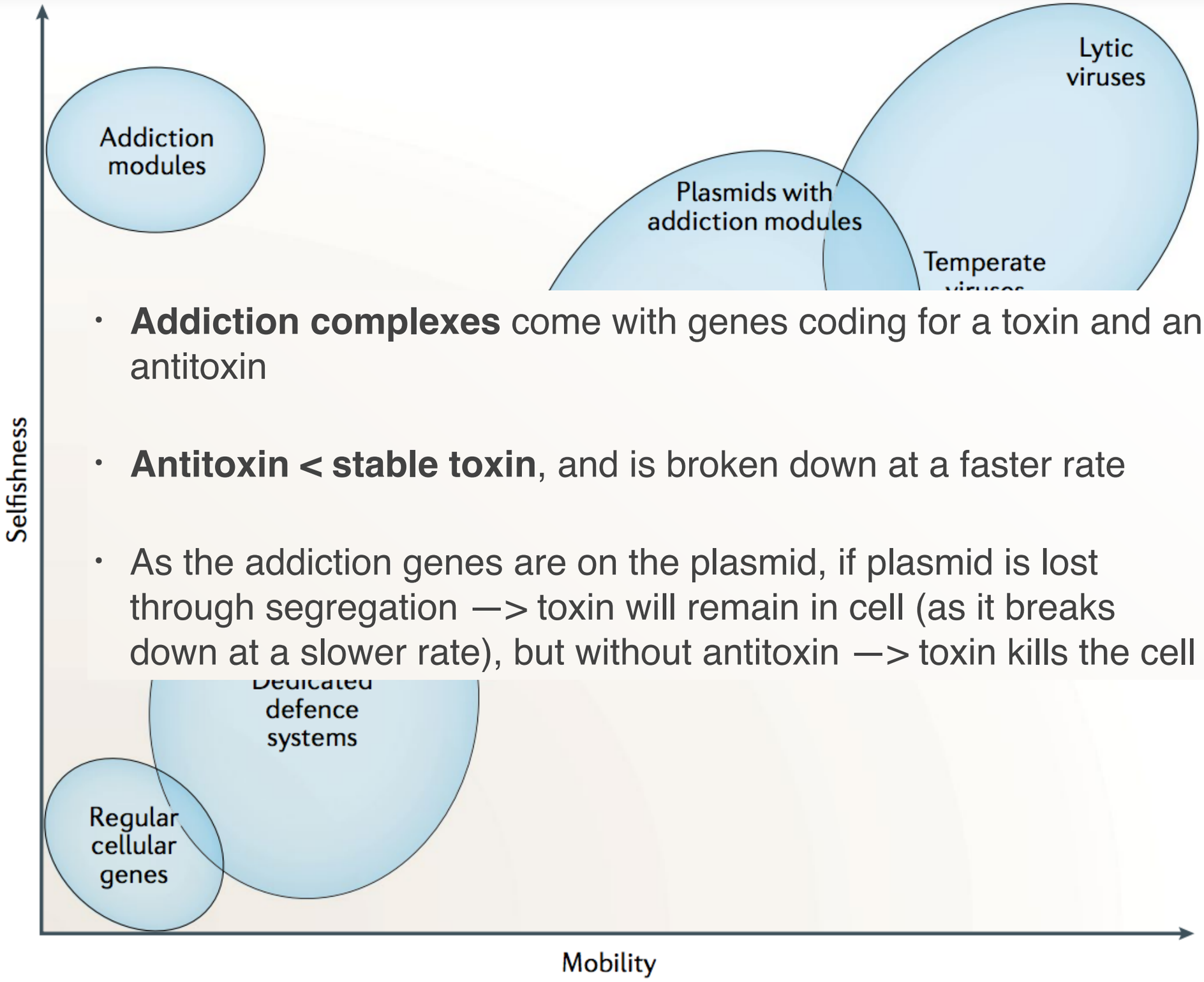
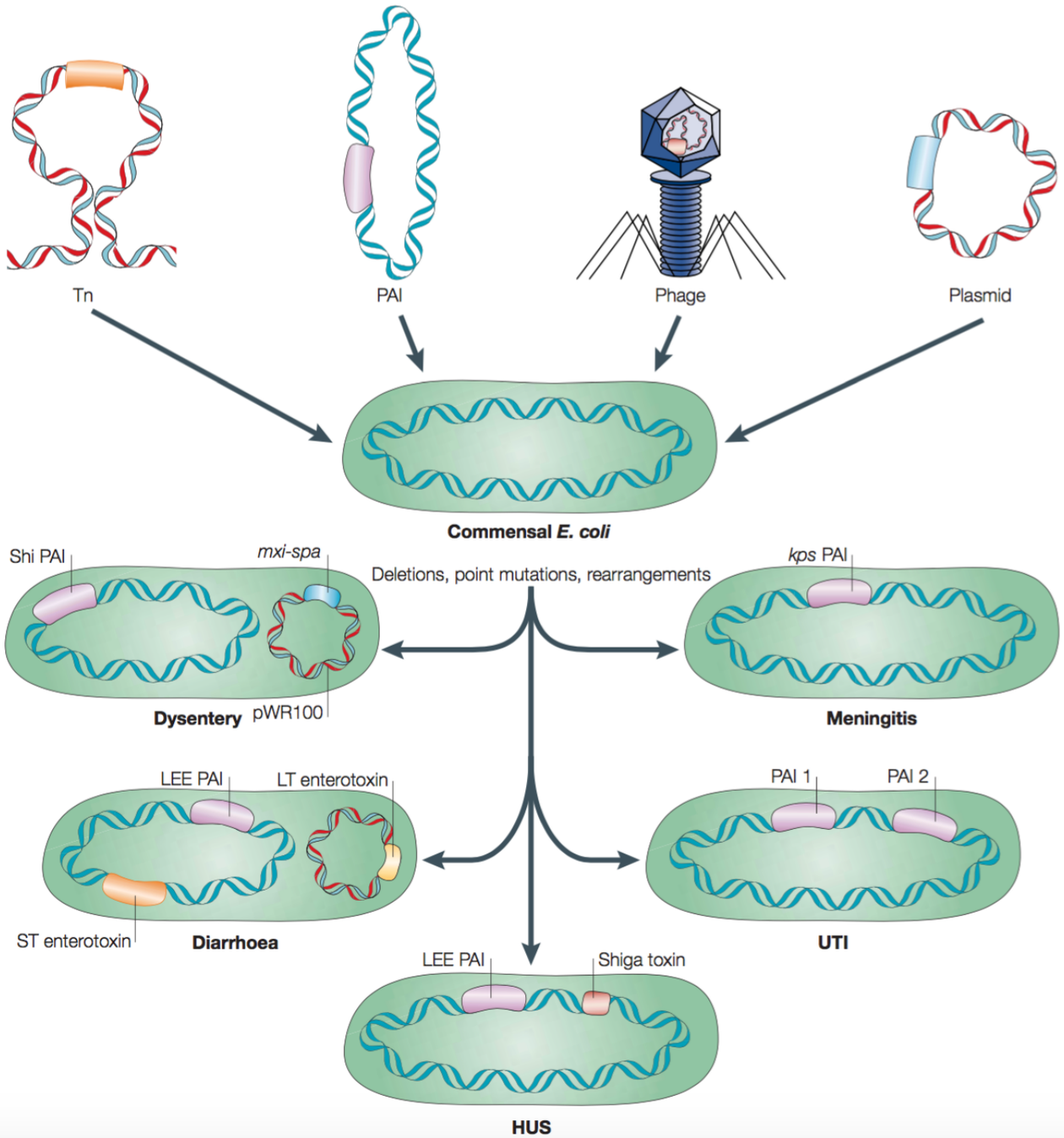


- **Addiction complexes** come with genes coding for a toxin and an antitoxin
- **Antitoxin < stable toxin**, and is broken down at a faster rate
- As the addiction genes are on the plasmid, if plasmid is lost through segregation —> toxin will remain in cell (as it breaks down at a slower rate), but without antitoxin —> toxin kills the cell

Koonin et al., 2020

Fig. 1 | **Distribution of mobile genetic elements and defence systems in the virtual space bounded by the axes of selfishness and mobility.** The plot is a conceptual diagram, and the positions of each class of elements are approximate. PICI, phage-inducible chromosomal island.

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)

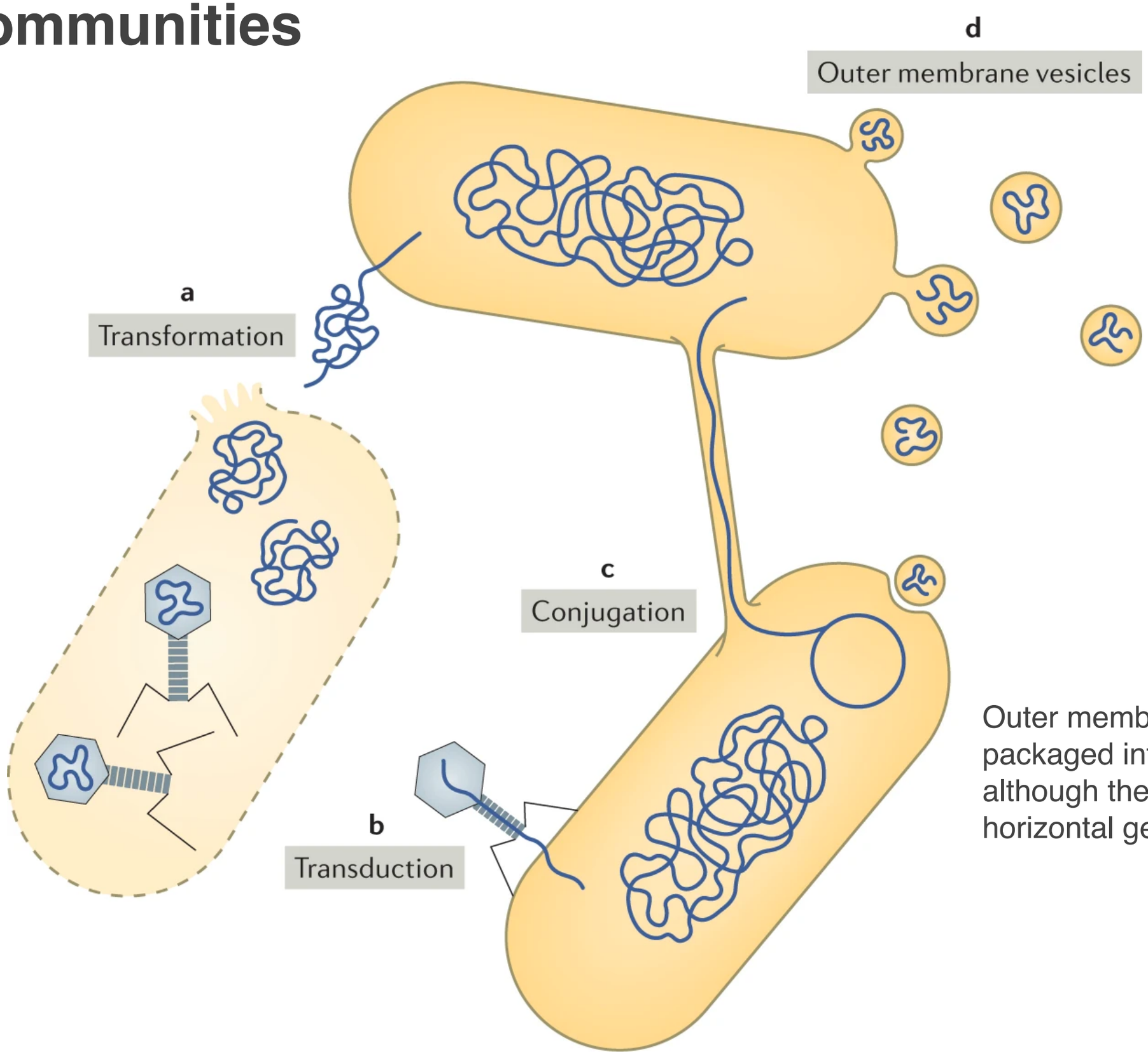
3 haemolytic uremic syndrome, hus (EHEC),

3 urinary tract infections, uti (UPEC)

4 meningitis (MNEC)

Kasper et al., 2004

General routes of horizontal gene transfer within natural communities

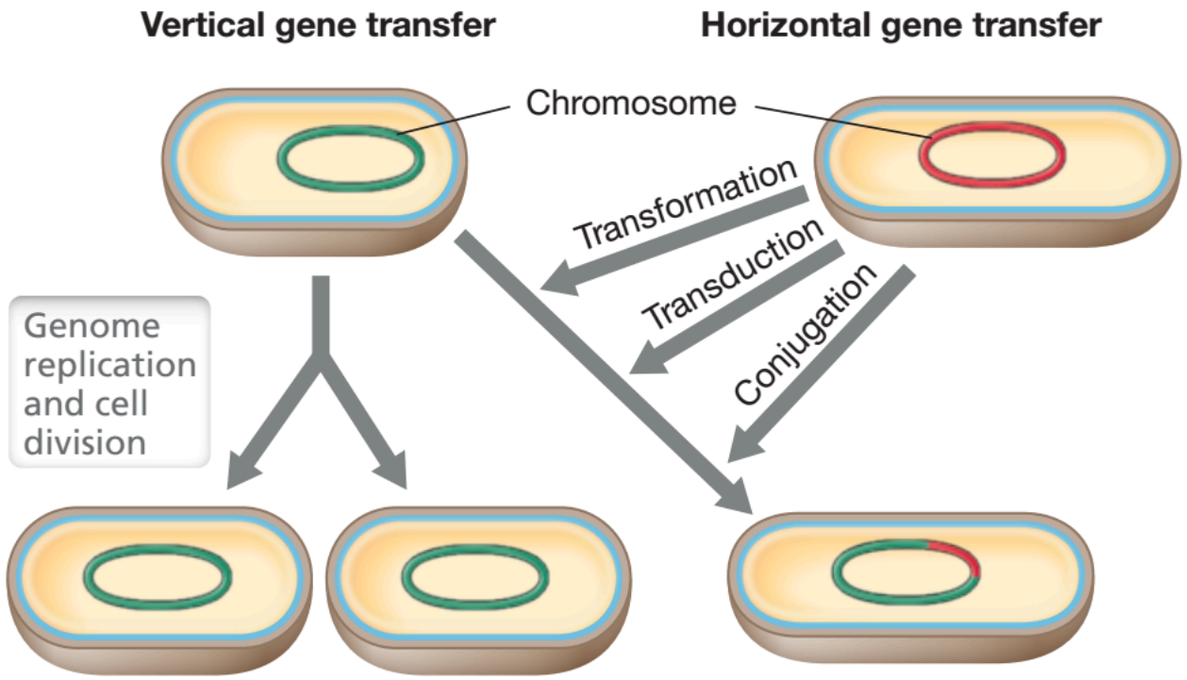


Brito, 2021

Outer membrane vesicles and DNA packaged into virus-like particles although their contribution to overall horizontal gene transfer is unknown

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

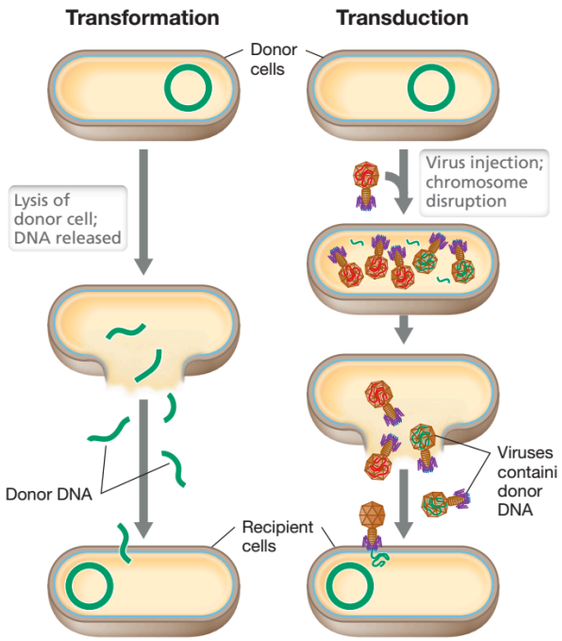
- Gene transfer from one cell to another by means other than the vertical process
- In prokaryotic cells, 3 HGT mechanisms: **transformation, transduction, conjugation**
- **HGT** can be detected in genomes once the genes have been annotated:
    a. Presence of genes that encode **proteins** typically 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

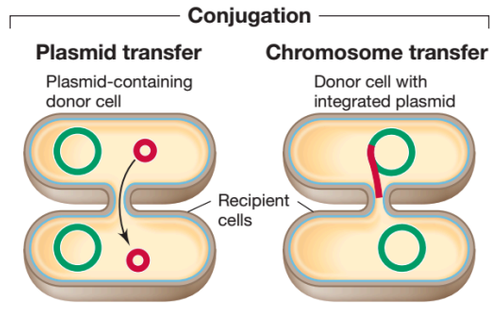
2. **Transduction**, in which DNA transfer is **mediated by a virus**

3. **Conjugation**, in which DNA transfer requires **cell-to-cell contact** and a conjugative plasmid in the donor cell



Madigan et al. 2018

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



- Fate of transferred DNA:

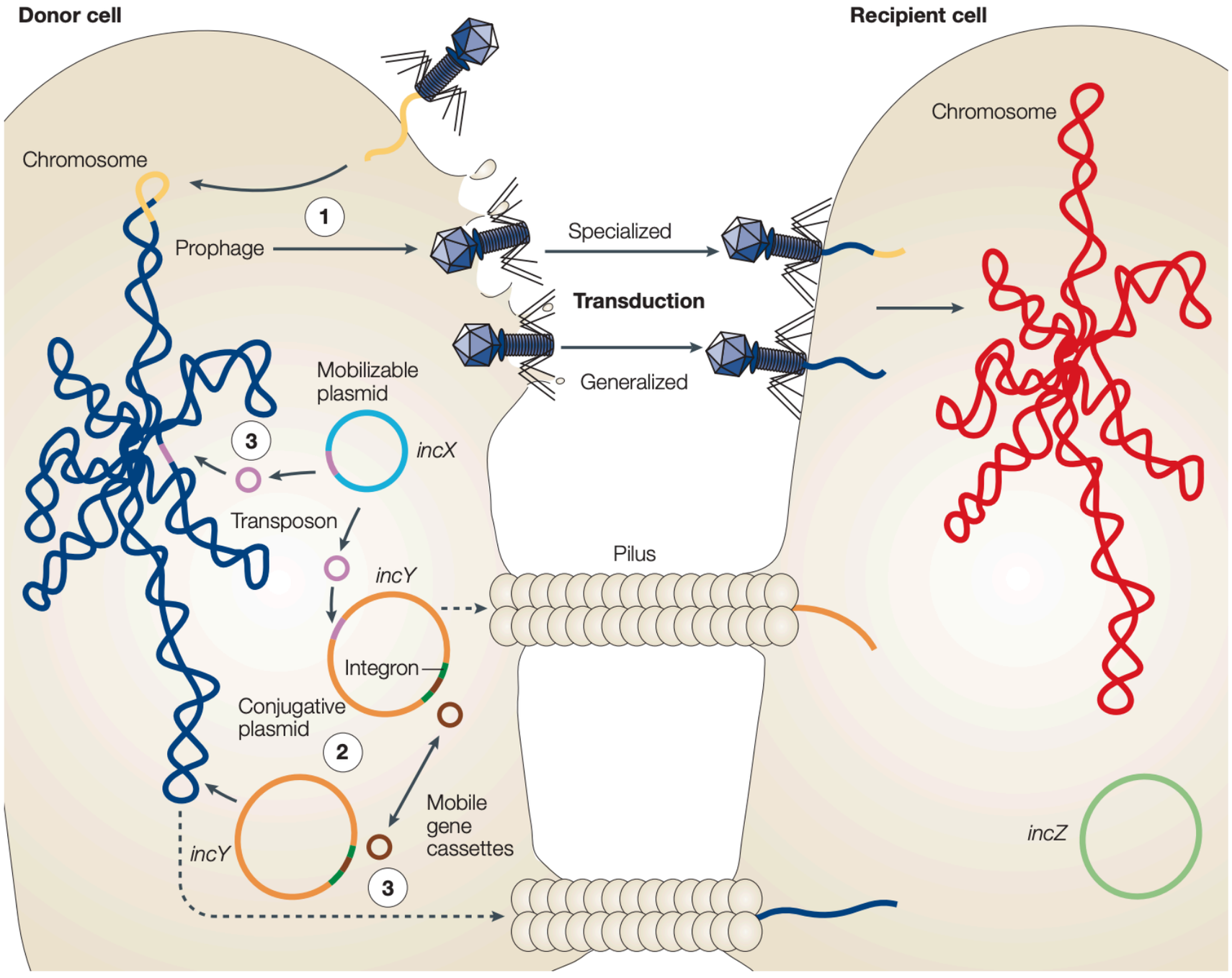
1. It may be degraded by the recipient cell's **restriction enzymes** or other DNA destruction systems

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

Mobile Genetic Elements

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

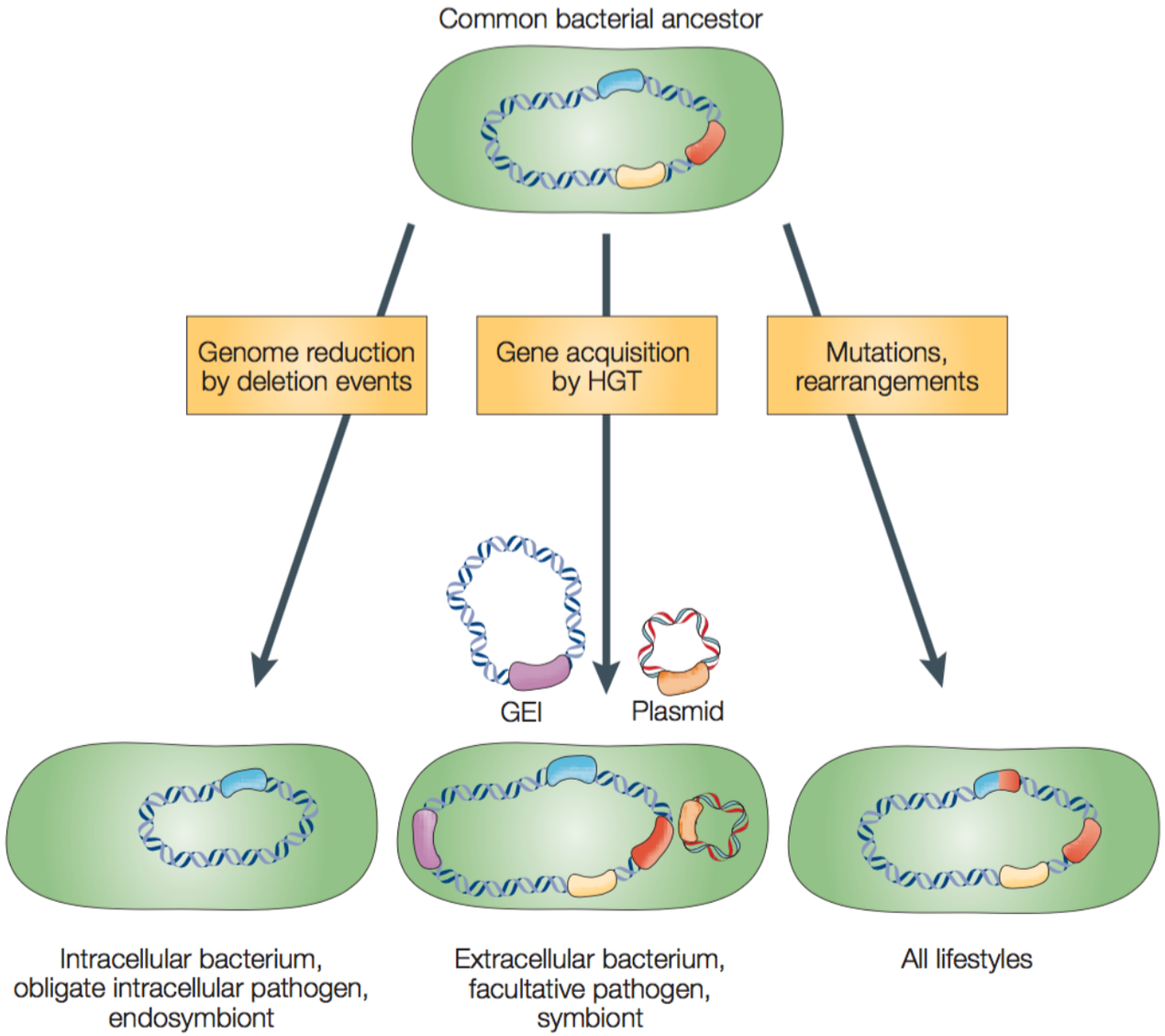
Evolution of bacterial variants by acquisition and loss of genetic information

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

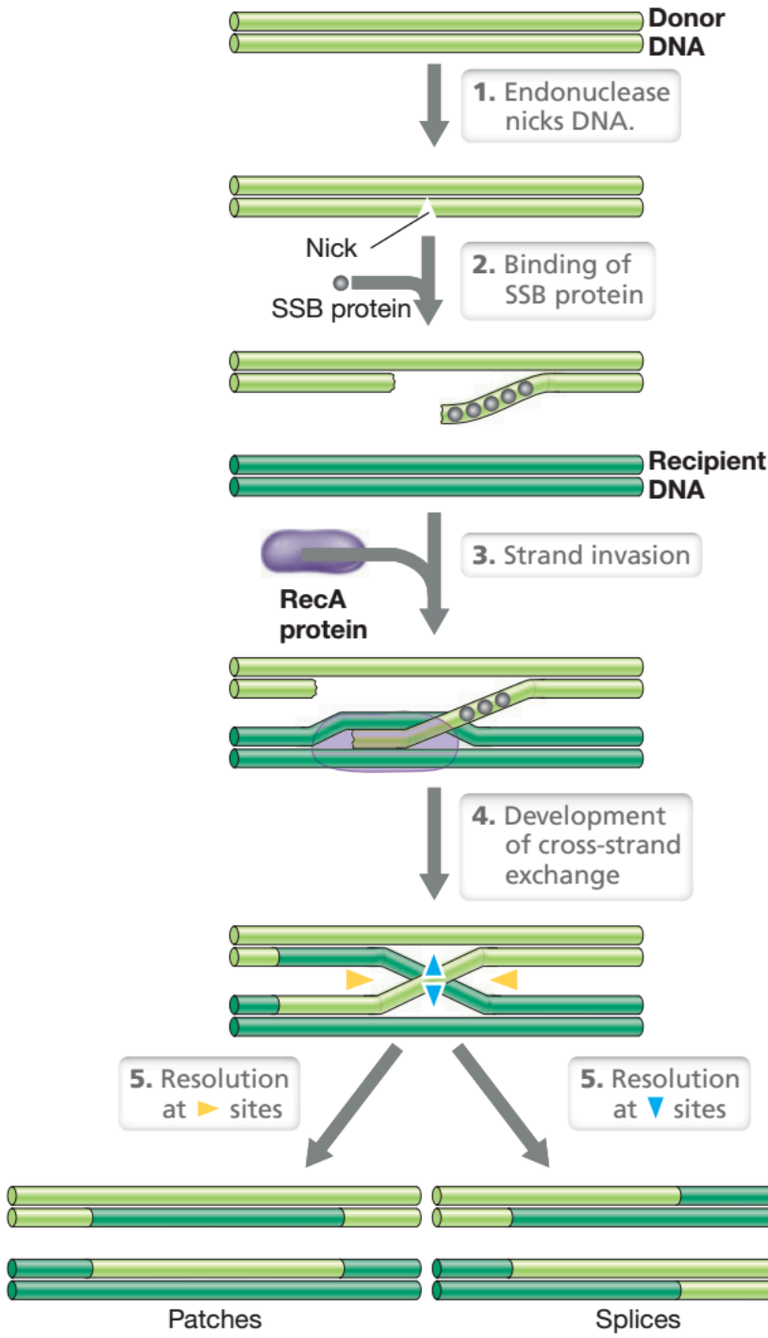
Point mutations and genetic rearrangements constantly contribute to evolution of new gene variants in **all types** of bacteria. HGT, horizontal gene transfer



Dobrindt et al., 2004

Recombination, I

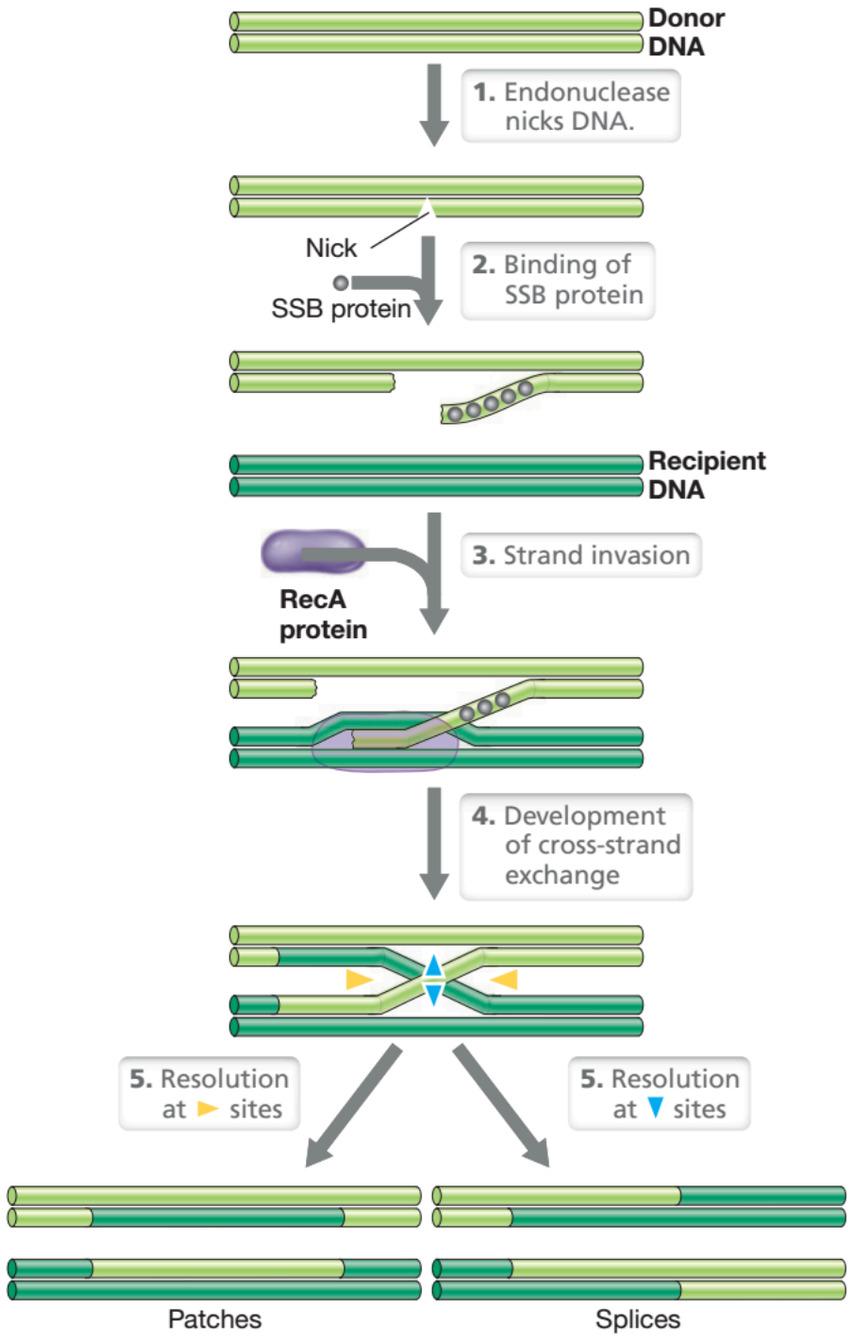
- **Recombination is the physical exchange of DNA** between genetic elements (structures that carry genet information) after HGT
- **Homologous recombination**, a process that results i genetic **exchange between homologous DNA sequences** from 2 different sources
- **Homologous DNA** sequences are those 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



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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

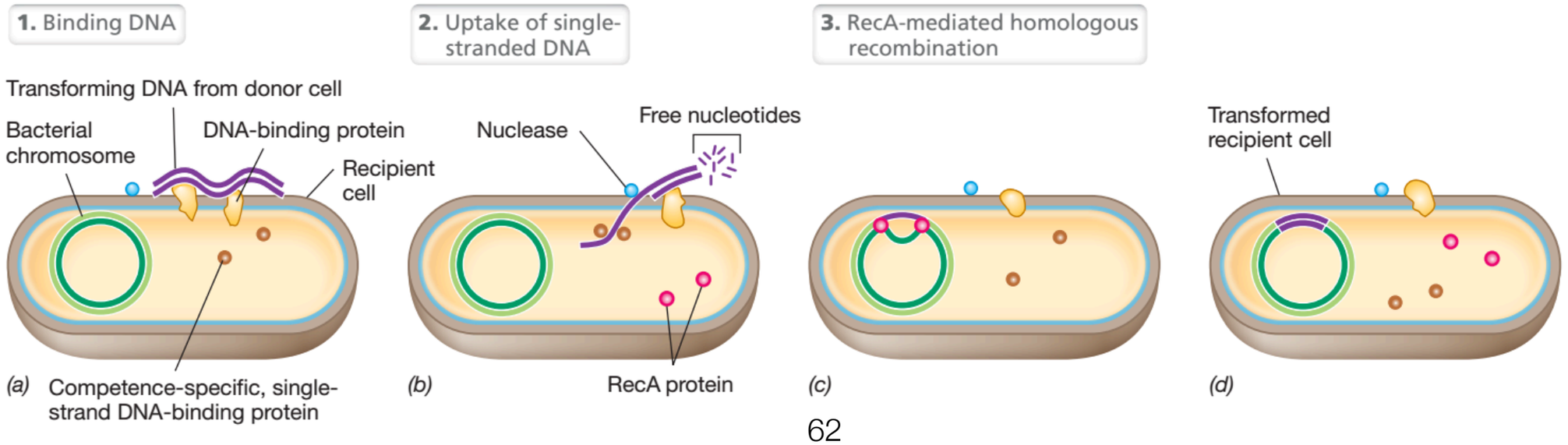
- **Ability of a cell to take up DNA and be transformed, is genetically determined**
- Competence is regulated: special proteins play a role in **DNA uptake and processing**
- **Competence-specific proteins**: membrane associated DNA-binding protein, a cell wall autolysin, and various nucleases
- Natural 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**



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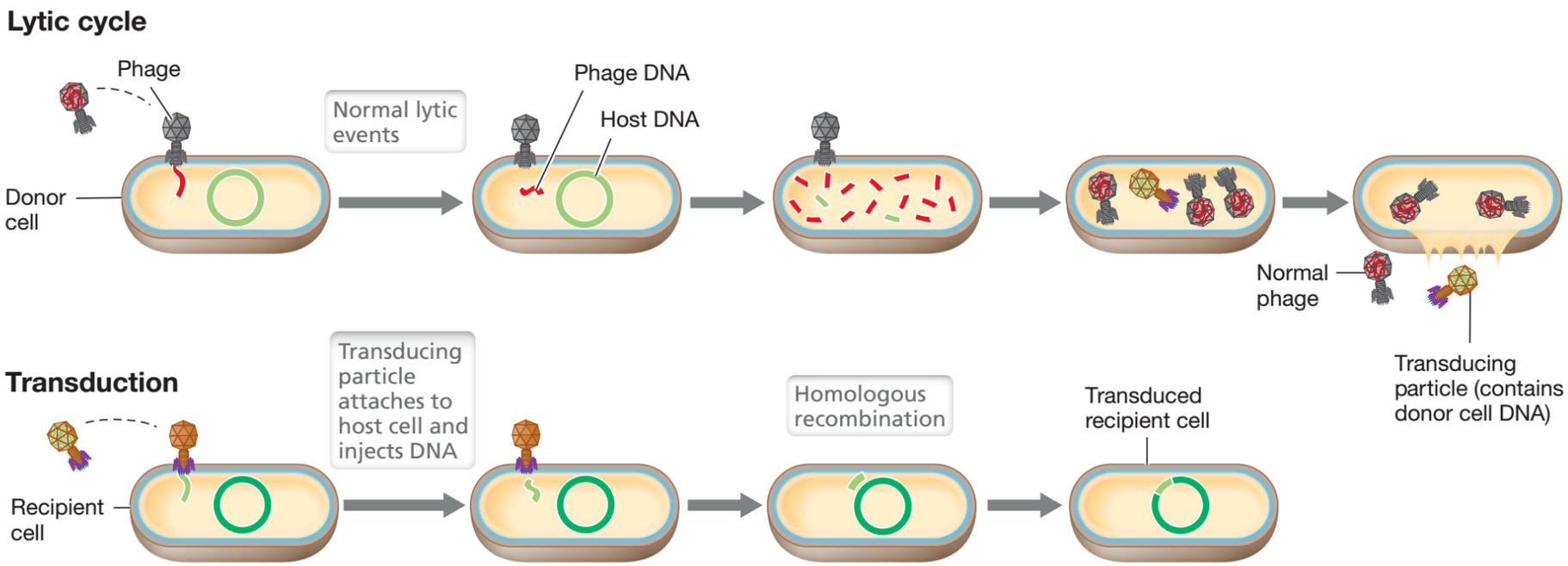
Transduction, I

- 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



- **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)

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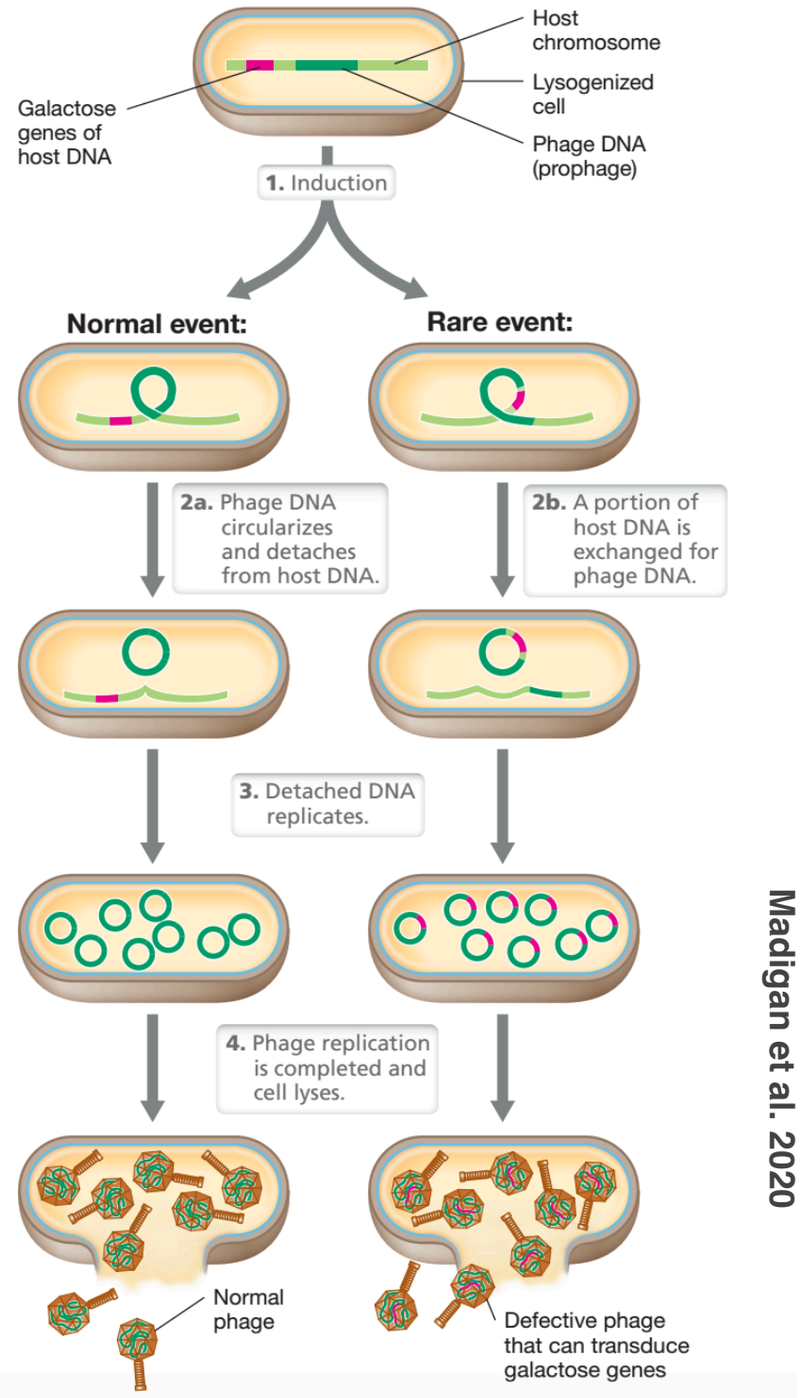
Transduction, III

- **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**



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**

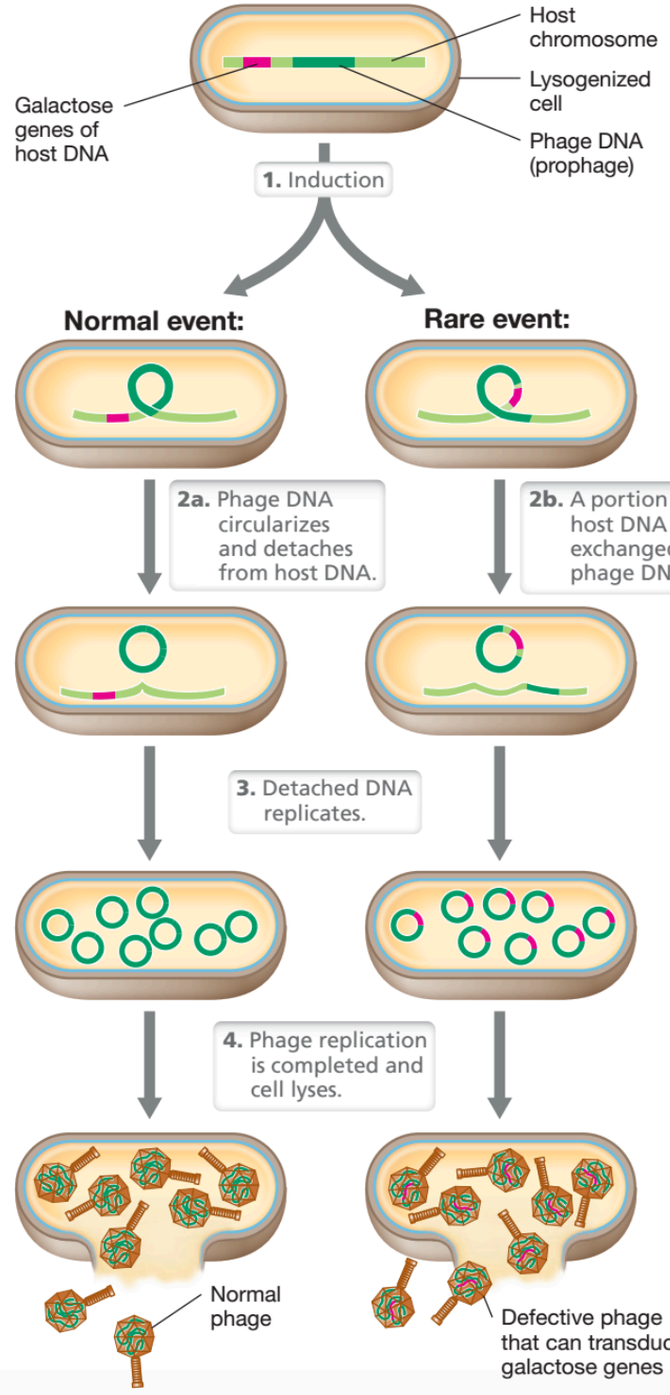
- However, other phenotypic changes unrelated to phage immunity are often observed in phage conversion of lysogenized cells:

1. Change in structure of a polysaccharide on the cell surface of *S. enterica (anatum)* w. bacteriophage ε15

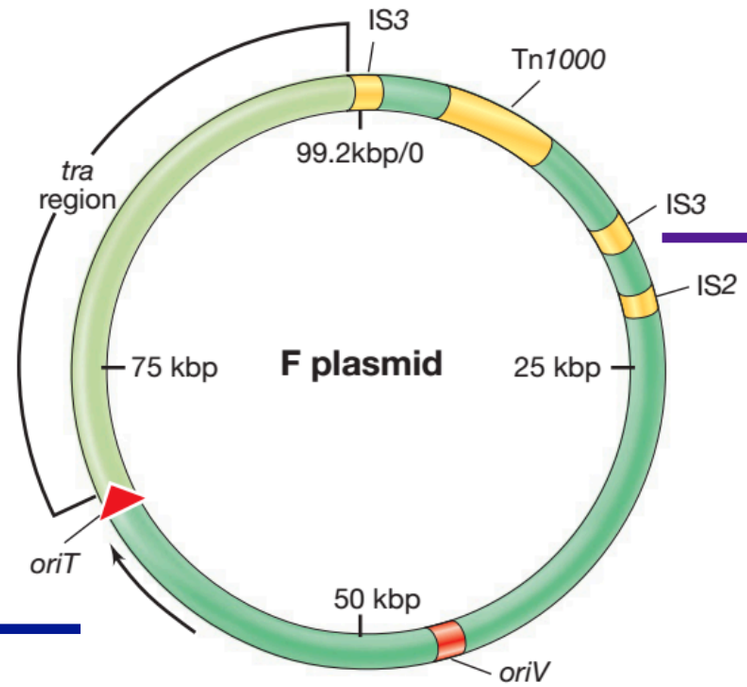
2. Conversion of non-toxinproducing strains of *Corynebacterium diphtheriae* (cause of disease diphtheria) to toxin-producing (pathogenic) strains w. bacteriophage b

- 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**



Conjugation, I



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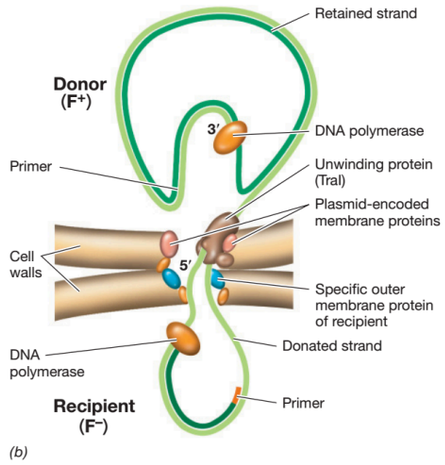
F plasmid

DNA transfer starts at oriT (origin of transfer) site

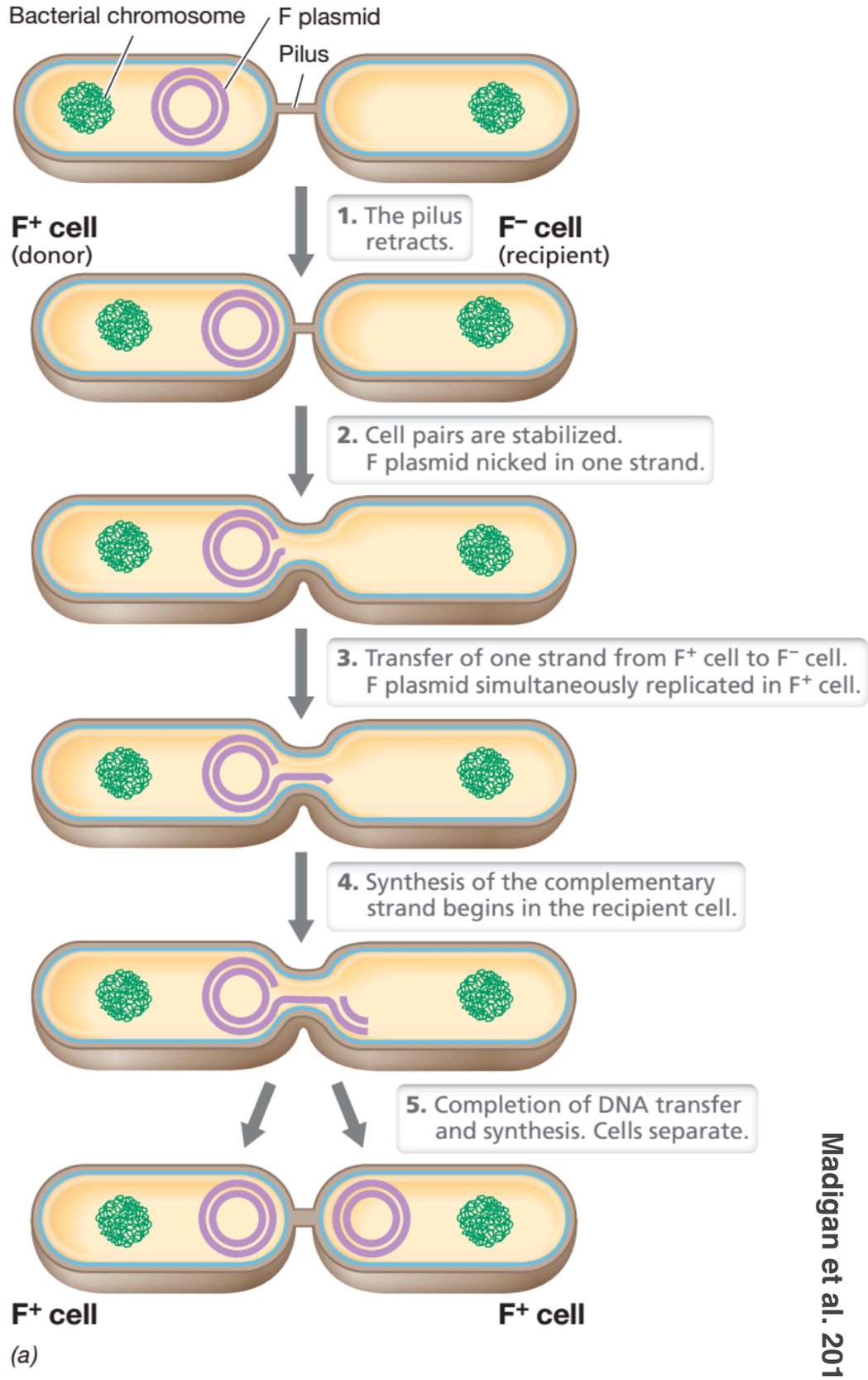
- Conjugation requires **cell-to-cell contact** (mating), plasmid encoded
- **Conjugative plasmids** use this mechanism to **transfer copies of themselves** and the **genes** they encode (e.g. antibiotic resistance), to new host cells
- Conjugation requires a **donor** cell, which contains the conjugative 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*



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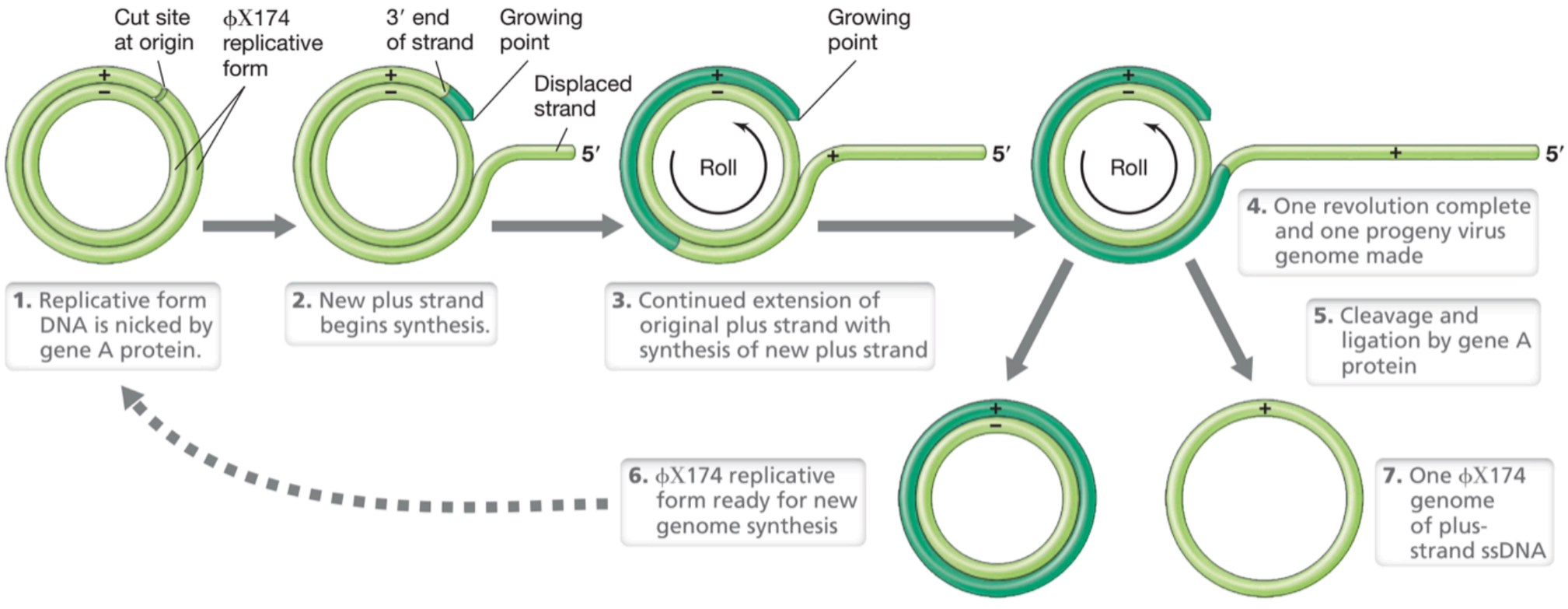
Rolling circle replication

- 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**



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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 o**nly part of the integrated F plasmid is transferred**
- **F- only if recombined w. chromosome can express new phenotypes**