L06c

Gene expression

Operon and its regulation

Diverse environmental stresses

Sensing the environment

Motility

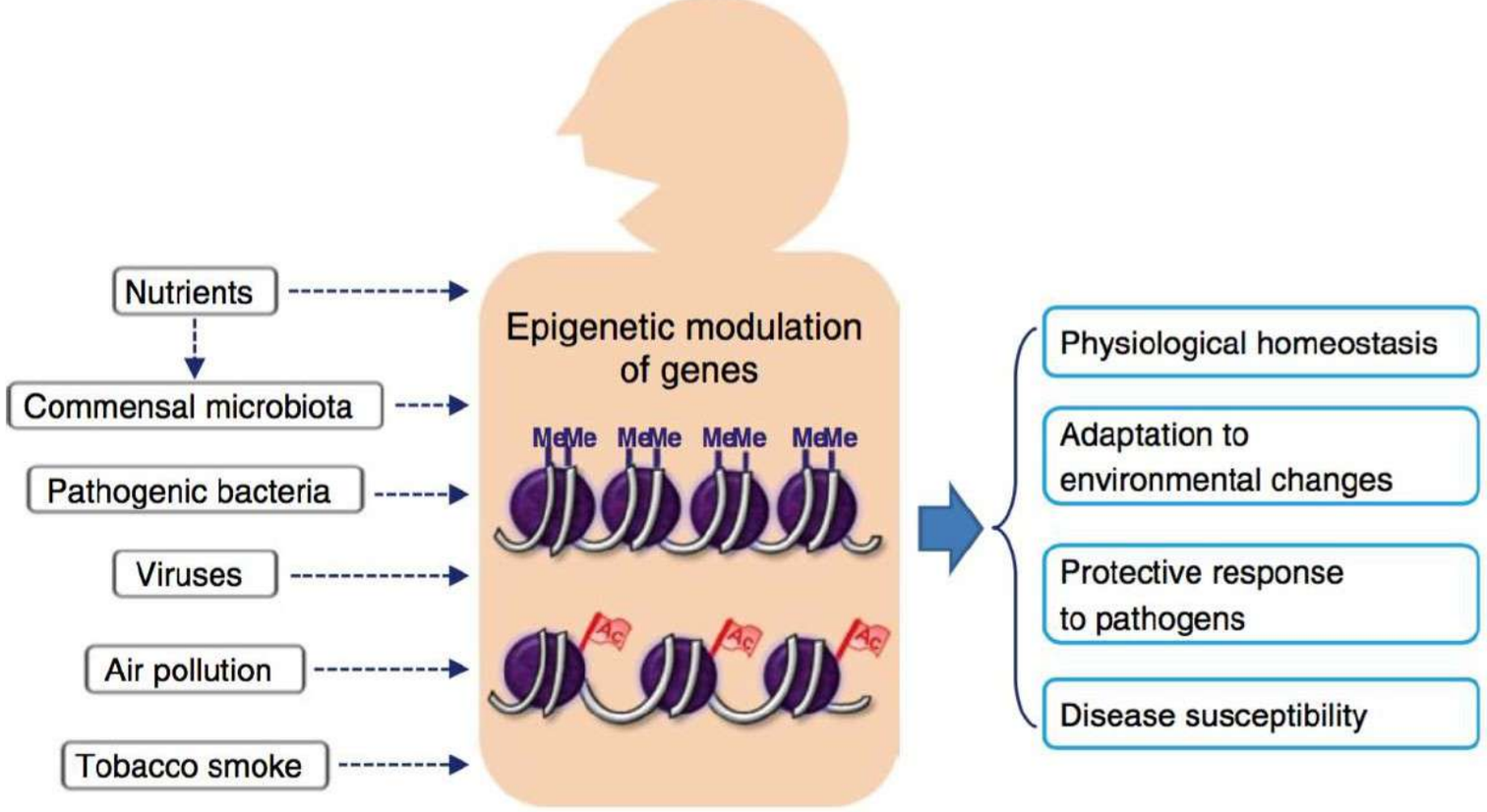
Epigenetics
Persistence-Tollerance-Resistance

Epigenetics

- The word “epigenetics” was originally coined by Conrad Waddington in 1942, referring to how genotypes give rise to phenotypes during development

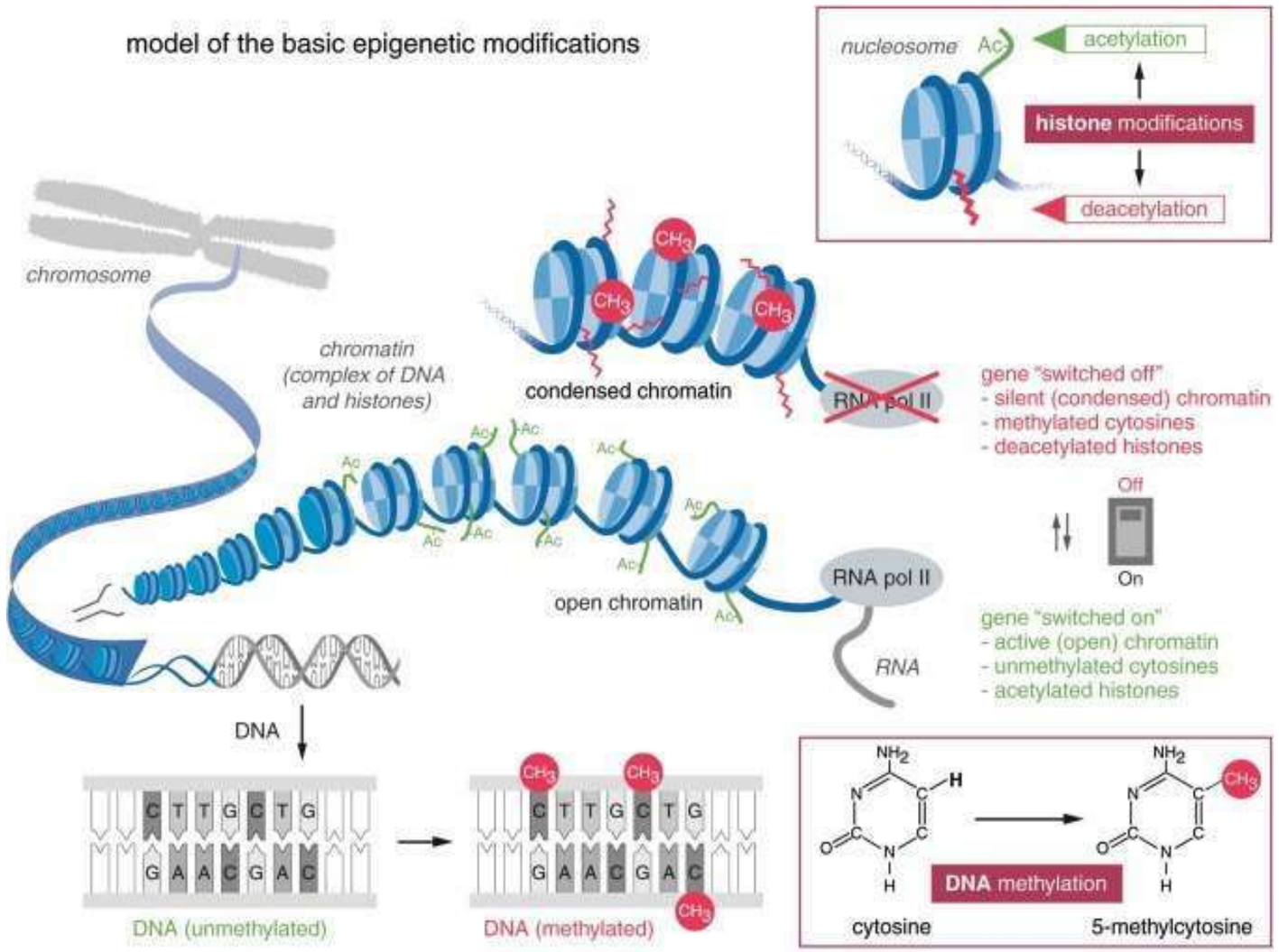
- Now we refer as the study of **phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression** that are **not dependent on changes to DNA sequence** (Deans and Maggert 2015)

- In Humans, gene expression is regulated prior to transcriptional initiation by the **chemical modification of DNA or the histone proteins** that together form chromatin



Takahashi, 2014

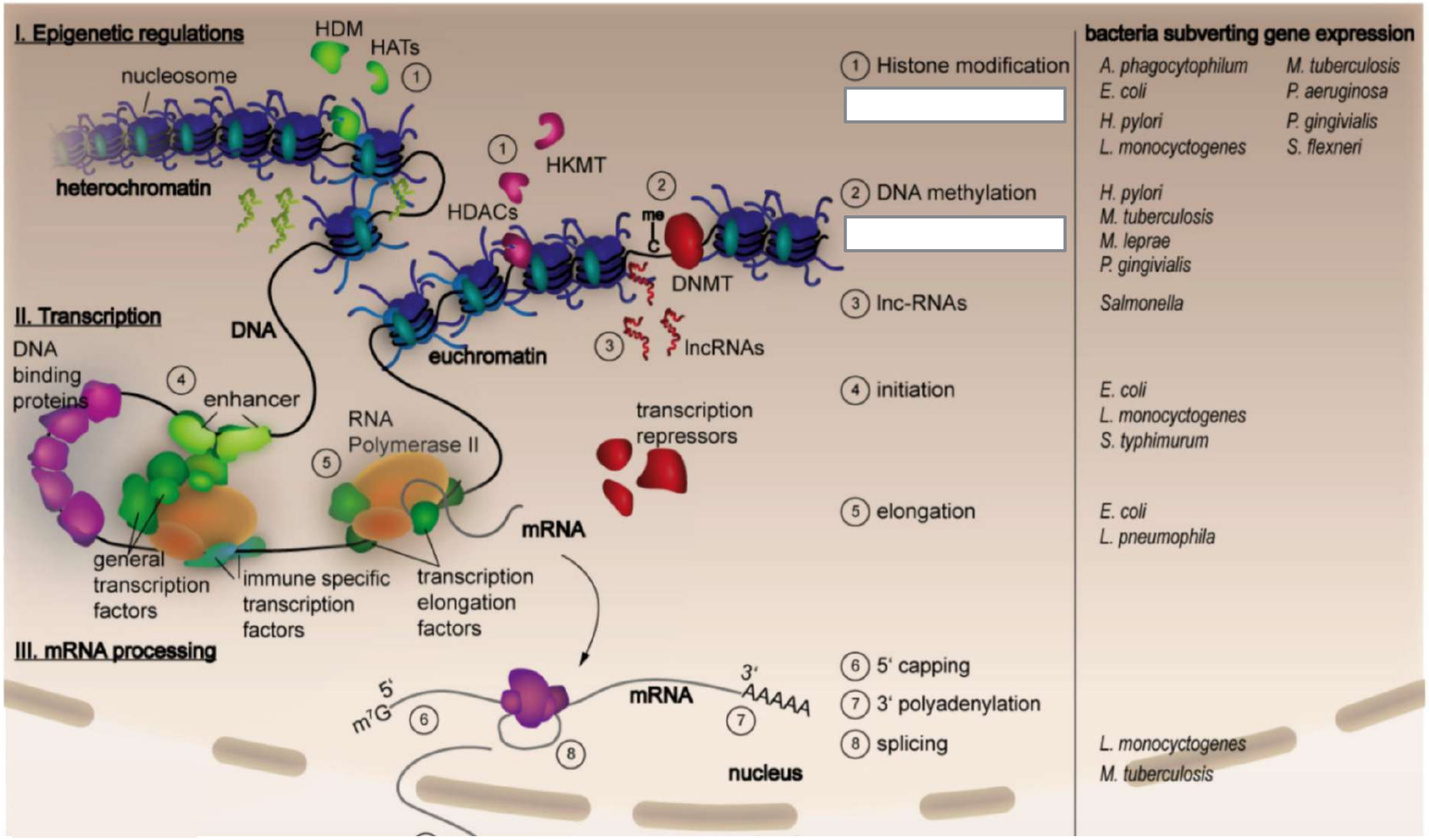
Epigenetic modifications of chromatin by DNA methylation and histone acetylation



Vilcinskas, 2015

- **Methyl** group transfer to **cytosine** — > 5-methylcytosine (m5C) pairs with guanosine m5C has different interactions with regulatory proteins
- **Chromatin structure** depends on net **charge** of core **histones**
- **Acetyl groups** promoting formation of *open* and **accessible** euchromatin vs **deacetylation** promoting the formation of *compact* and **inaccessible** heterochromatin

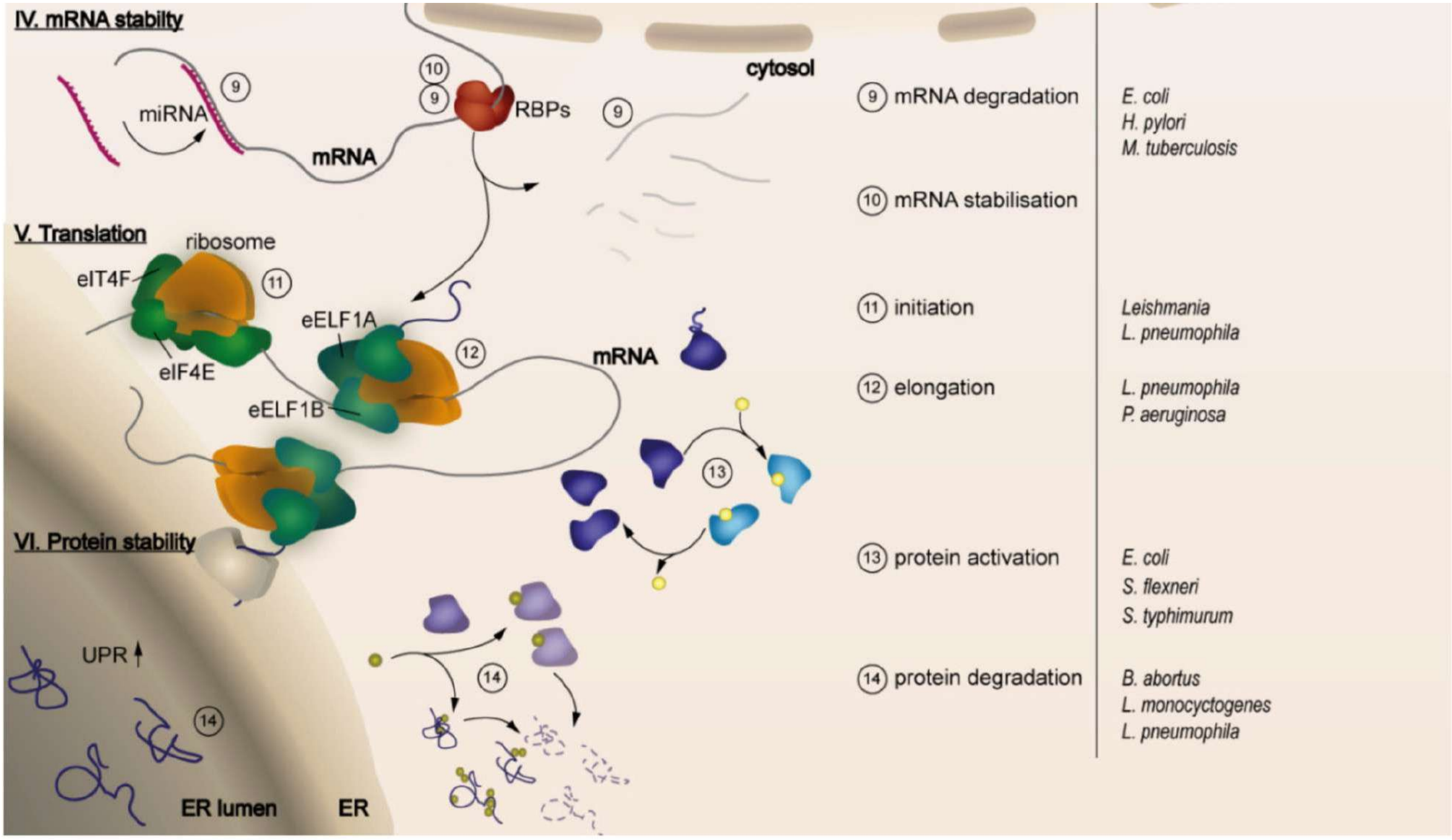
Bacteria manipulate host gene expression during infection, I



Denzner et al., 2020

Bacteria evolved many strategies to survive and persist within host cells

Bacteria manipulate host gene expression during infection, II



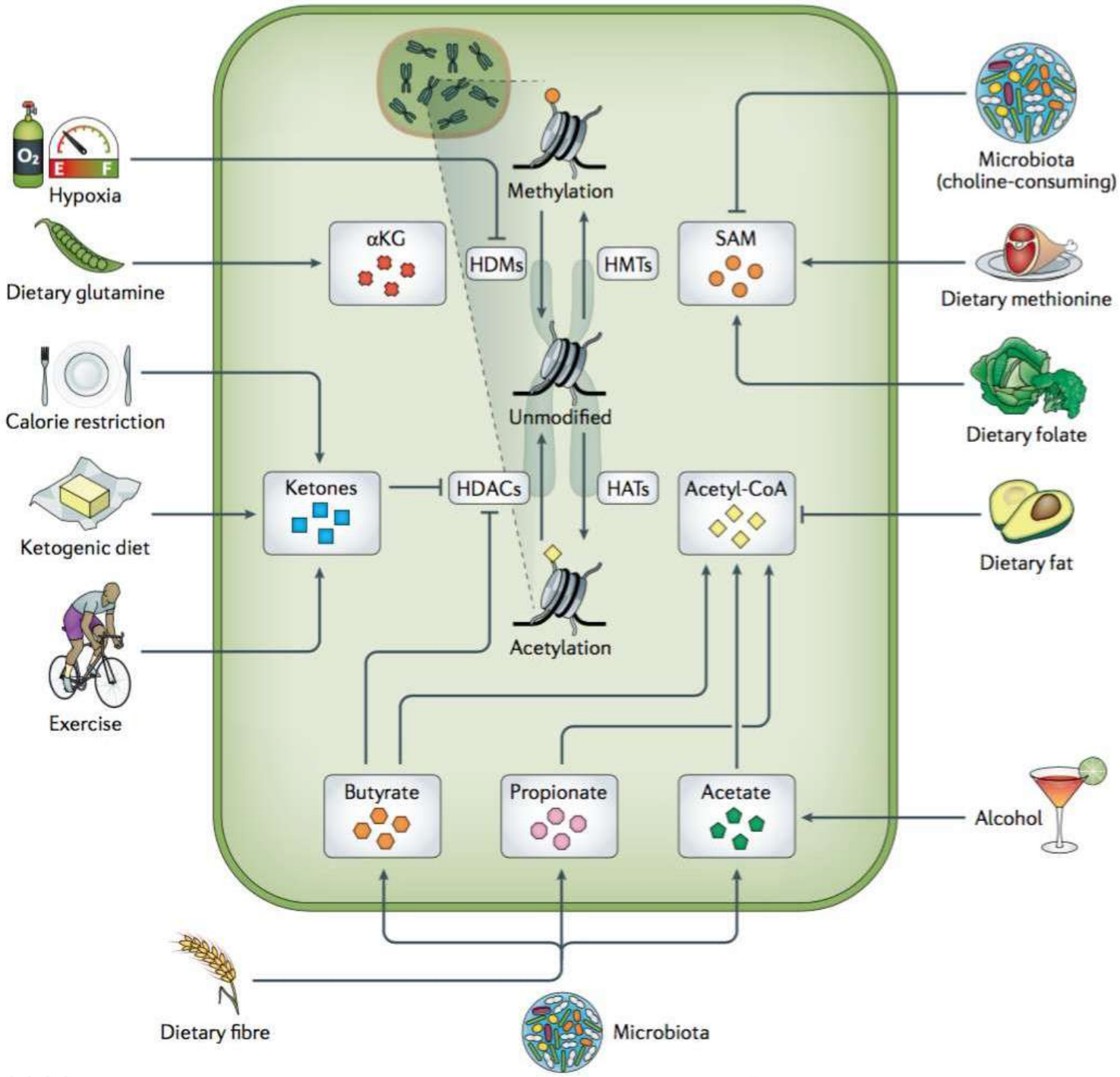
Denzner et al., 2020

Bacteria, small Eukaryotes and Viruses influencing host via epigenetic attack

Microbe	Factor	Effect on the host
Listeria monocytogenes	LntA	Inhibition of binding of a chromatin silencing complex to the promoters of interferon-stimulated genes
		Increase in IL-8 gene expression by inducing histone modifications through activation of MAPK signaling pathway
	LLO	Dephosphorylation of histone H3 through induction of K^+ efflux
Chlamydia trachomatis	NUE	Methylation of histones
Legionella pneumophila	RomA	Methylation of histones (H3K14 trimethylation)
	Flagellin	Increase in IL-8 gene expression by inducing histone modifications through activation of a signaling cascade
Helicobacter pylori		Silencing selected promoters by inducing DNA methylation
		Induction of histone modifications
		Regulation of miRNA expression
Bacteroides vulgatus		Induction of histone modifications through a signaling cascade
Wolbachia		Interference with genetic imprinting by altering methylation patterns
Bifidobacterium breve, Lactobacillus rhamnosus GG		Decrease in LPS-induced IL-17 and IL-23 production by suppressing histone acetylation
Porphyromonas gingivalis		Reactivate latent HIV-1 integrated in the host genome as proviral DNA copies by butyrate-meditated HDAC inhibition
Influenza virus	NS1	Suppression of antiviral protein production by hijacking a transcription elongation factor through a region similar to H3 histone tail
Epstein–Barr virus	LMP1	Silencing of E-cadherin promoter by upregulating Dnmt1, 3A, 3B through the JNK-AP-1 pathway
Human adenovirus	E1A	Up-regulation of Dnmt1 by activation of E2F
		Activation of Dnmt1 by associating with Dnmt1
Hepatitis B virus	pX (HBx)	Silencing of tumor suppressor genes by up-regulating Dnmt1 through the cyclin D1-CDK4/6-pRb-E2F1 and p38 MAPK pathways
HIV	Early expressed proteins	Silencing of IFN-γ promoter by up-regulating Dnmt1 through the AP-1 pathway

Takahashi, 2014

Influences of environmental factors on histone acetylation and methylation via microbiome



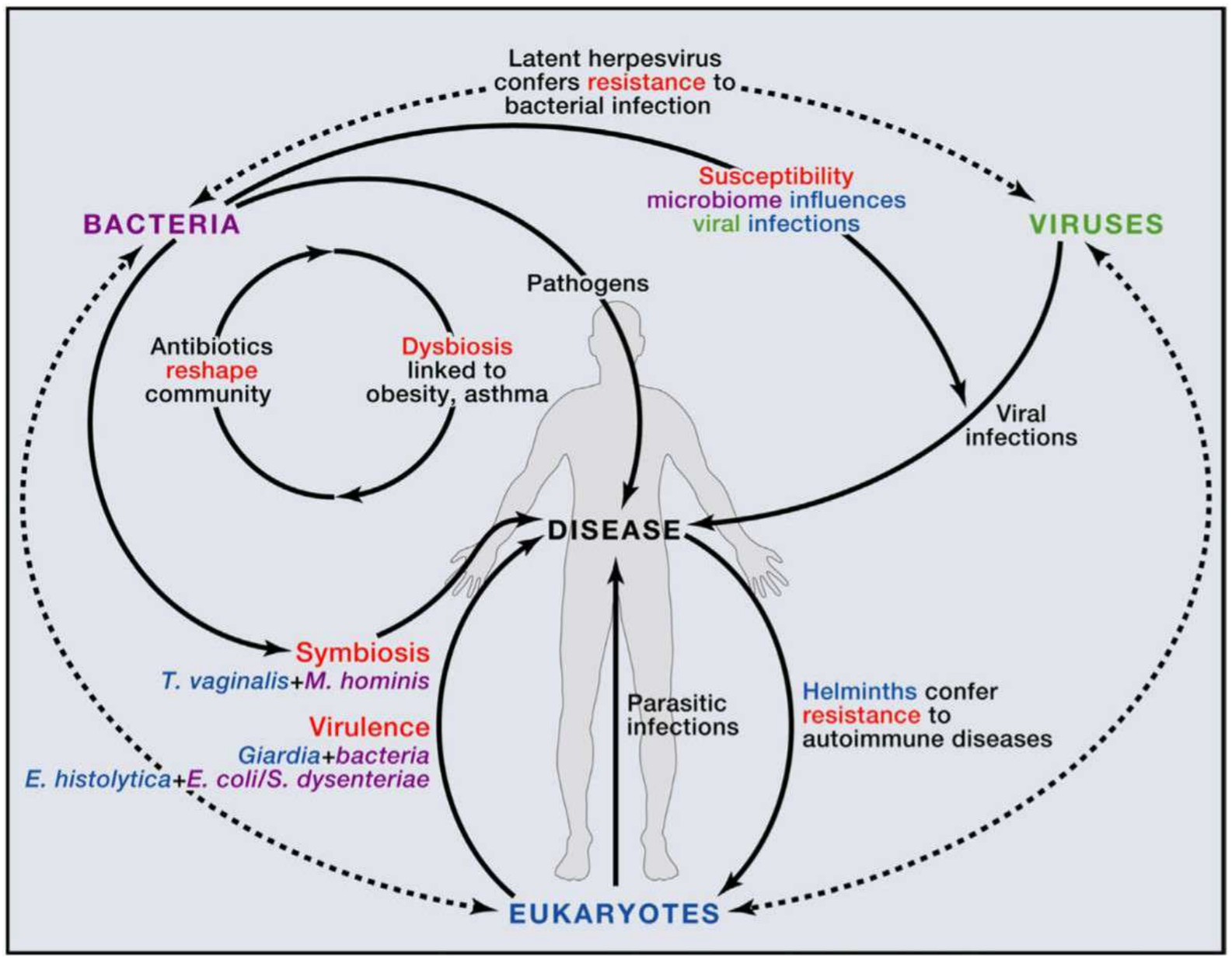
S-adenosylmethionine (**SAM**) and acetyl-CoA, that are used by histone methyltransferases (HMTs) and histone acetyltransferases (HATs)

The activity of histone demethylases (**HDMs**) is supported by α-ketoglutarate (αKG), which can be derived from dietary glutamine, and is inhibited by the limited oxygen availability during hypoxia

Ketone bodies and short-chain fatty acids (SCFAs) such as acetate, propionate and **butyrate** can provide **acyl-CoA** precursors for histone acylation, while also directly inhibiting the activity of histone deacetylases (HDACs)

Dai et al., 2020

Why is important to consider epigenetics?

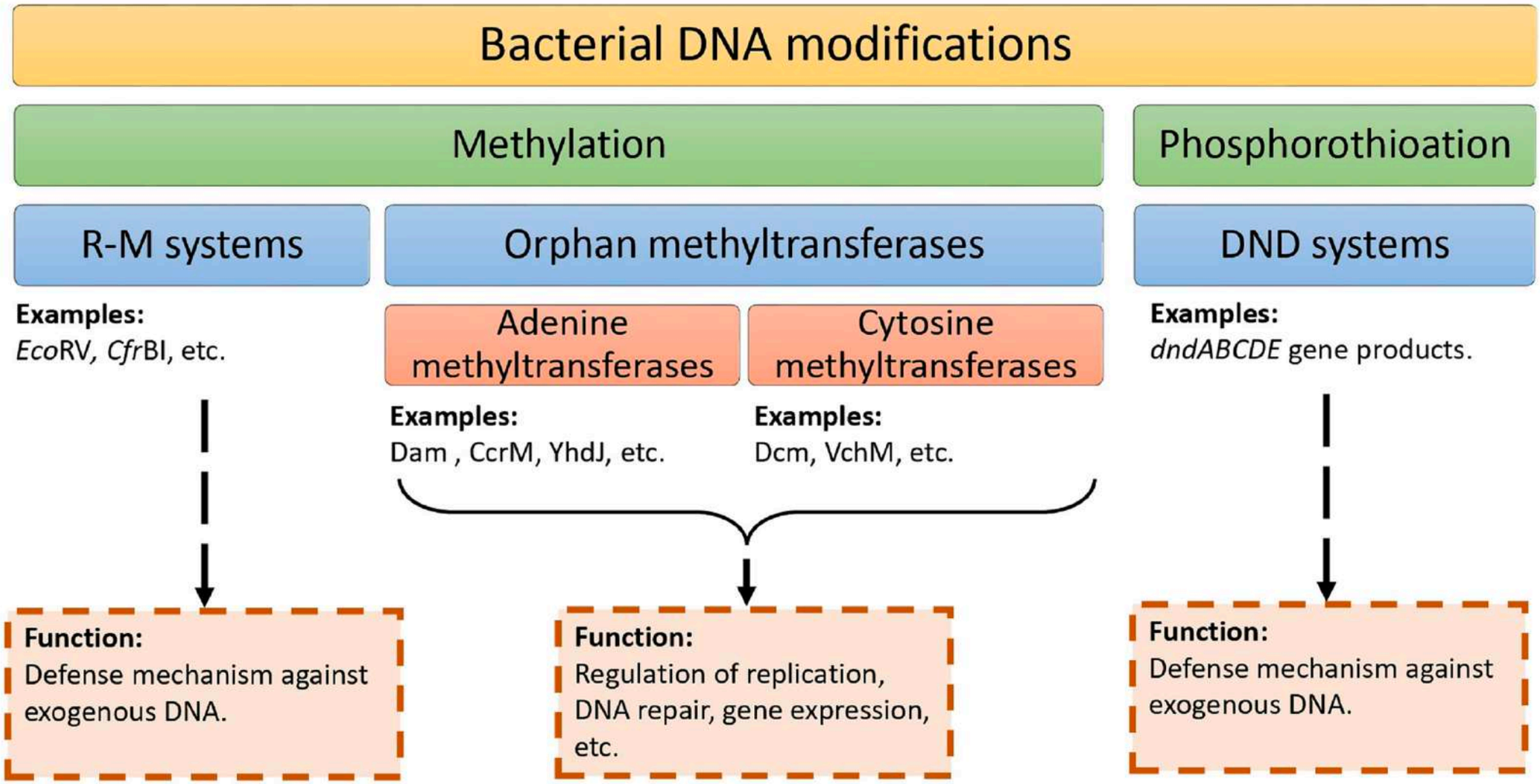


To fully understand the **microbial interactions in human health and disease** — > new medicine and societal norms

To fully understand the microbial **interactions at the microscale** in our world — > modeling and protecting environment

Watson & Søreide, 2017

Epigenetics in Bacteria



There are two broad classes of bacterial DNA modifications:

A) Methylation of adenines and cytosines

B) Phosphorothioation of the DNA backbone, where a nonbridging oxygen gets replaced by sulfur

Bacterial DNA methylation is mediated by enzymes belonging to:

I) Restriction-modification (R-M) systems

II) Orphan methyltransferases

Definitions, I

- **Epigenome**: complete record of all chemical modifications to DNA
- Epigenome with the epitranscriptome (chemical modifications of RNA) and epiproteome (chemical modifications of proteins), makes up the **epi-ome**
- **Methylome**: complete record of all methyl modifications to either DNA, RNA, or proteins in a particular cell or organism

Definitions, II

- **DNA methyltransferase (MT-ases)**: family of enzymes that catalyze the **transfer** of a **methyl group** from an S-adenosyl-Lmethionine (AdoMet) donor to DNA

- **Restriction-modification (R-M) systems** almost ubiquitous in prokaryotes

- R-M consist of a **DNA methyltransferase** that methylates a specific target sequence in the host genome and a **cognate restriction endonuclease** that cleaves unmethylated or inappropriately methylated targets from exogenous DNA

- R-M system recognises “self” from “non-self” –> defence mechanism

Definitions, III

- **Orphan** or **solitary methyltransferase** *doesn't* have the cognate restriction **endonuclease**

- Orphan methyltranseferases are conserved as other genes at the genus level

- Possibly origin from R-M system with gene loss

- Methyl-directed mismatch DNA repair and regulation of chromosomal replication

- Cell cycle regulation

A second derivative in epigenetics: increase in diversity of adaptive strategies

- MTases can generate **phenotypic lineages**, which enables **division of labour** in a community or prepares the community for future changes in the environment (bet-hedging)

- **Bistability**: the state existing in a **clonal population with different phenotypes**

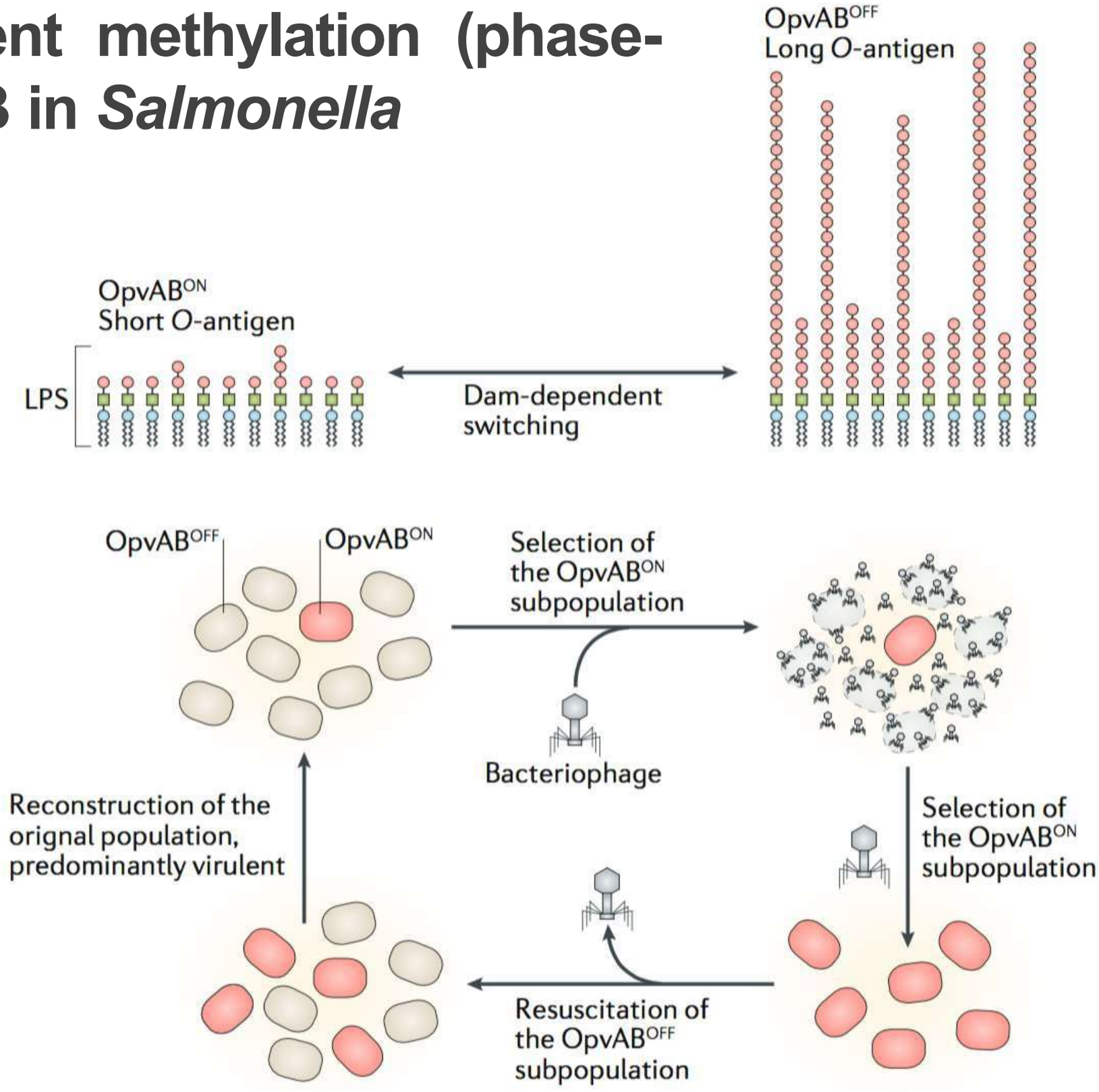
- **Phase variation**: a strategy to generate phenotypic diversity in a bacterial population in the absence of selection. It involves reversible, high-frequency ON/OFF switching of gene expression, showing programmed reversion

- Due to genetic rearrangements, DNA MTase can generate a **distinct methylation pattern in genome —>** which results in **different gene expression profiles** and produces **lineages with different** (virulence, antibiotic “tolerance and persistence”) capacities

Formation of subpopulations controlled by Dam-dependent methylation (phase-variation): OpvAB in *Salmonella*

Shortening of the O-antigen renders the OpvABON lineage avirulent but resistant to bacteriophages

When the phage challenge ceases, OpvABOFF cells produced by phase variation will survive, and virulence will be regained

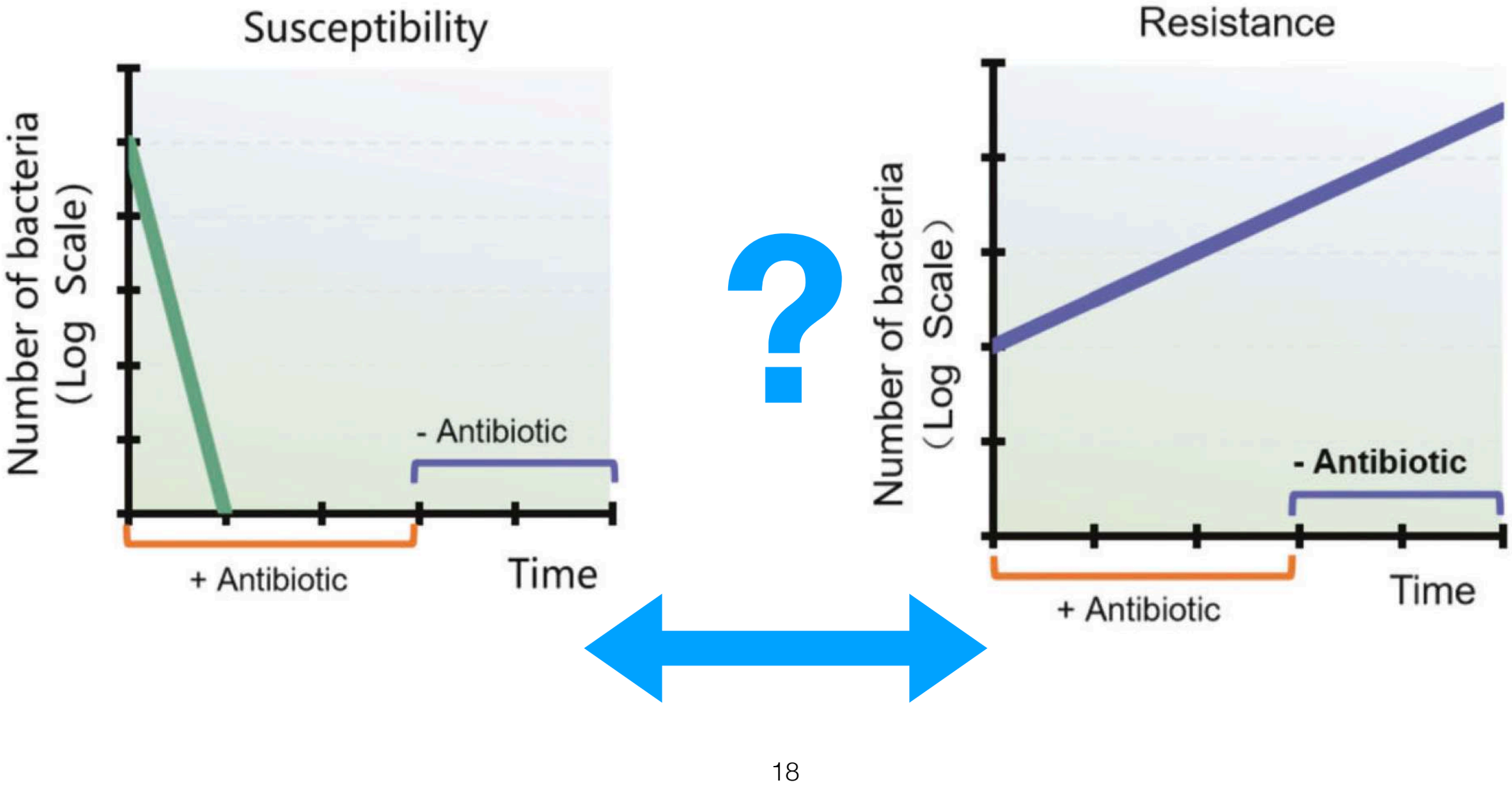


Sánchez-Romero & Casadesús, 2019

Box 1 | The epigenomes of eukaryotes and bacteria

- In eukaryotes, epigenetic modification of the genome involves DNA methylation[3] and histone modification[4]. Bacteria lack histones, and epigenetic control relies on DNA methylation only[6].
- In eukaryotes, de novo and maintenance forms of DNA methylation are performed by separate enzymes[2]. Bacterial DNA methyltransferases have both de novo and maintenance activities[37].
- In eukaryotes, two main mechanisms exist to erase DNA methylation marks: active demethylation by dedicated proteins (Tet enzymes), and passive demethylation by the hindrance of DNA methylase activity upon DNA replication[35]. In bacteria, DNA demethylation is usually passive[66], and the relevance of active demethylation by DNA repair remains to be evaluated[82].
- In both bacteria and eukaryotes, transcriptional repression by DNA methylation is common[3,6]. Transcriptional activation of bacterial genes under DNA methylation control often involves demethylation (partial or complete, single- or double-stranded) of promoters or regulatory regions[57,72,89,90,94,158].
- The methylated base typically involved in the control of eukaryotic transcription is C^5-methyl-cytosine[3], whereas in bacteria it is often N^6-methyl-adenine[7,14]. However, direct control of bacterial transcription by C^5-methyl-cytosine has been demonstrated recently[126]. Transcriptional control by N^4-methyl-cytosine may also exist[130].
- In multicellular eukaryotes, the DNA methylation pattern of the genome is reprogrammed during gametogenesis and during early embryonic development[2]. In bacteria, reprogramming does not occur, and the DNA methylation pattern can be transmitted unaltered across generations. However, the acquisition and loss of DNA methyltransferase genes[41] and recombinational shuffling of DNA methyltransferase domains[27,33,143] can produce novel methylation patterns in bacterial genomes.
- In both bacteria and eukaryotes, DNA methylation controls the formation of phenotypic variants of genetically identical cells. However, DNA methylation-dependent formation of bacterial cell lineages can show programmed reversion (phase variation)[15,27,93,111].

256 shades of grey: Antibiotic *vs.* Microbe



Niu et al., 2024

Antibiotic: Bacteriostatic *vs.* Bactericidal, I

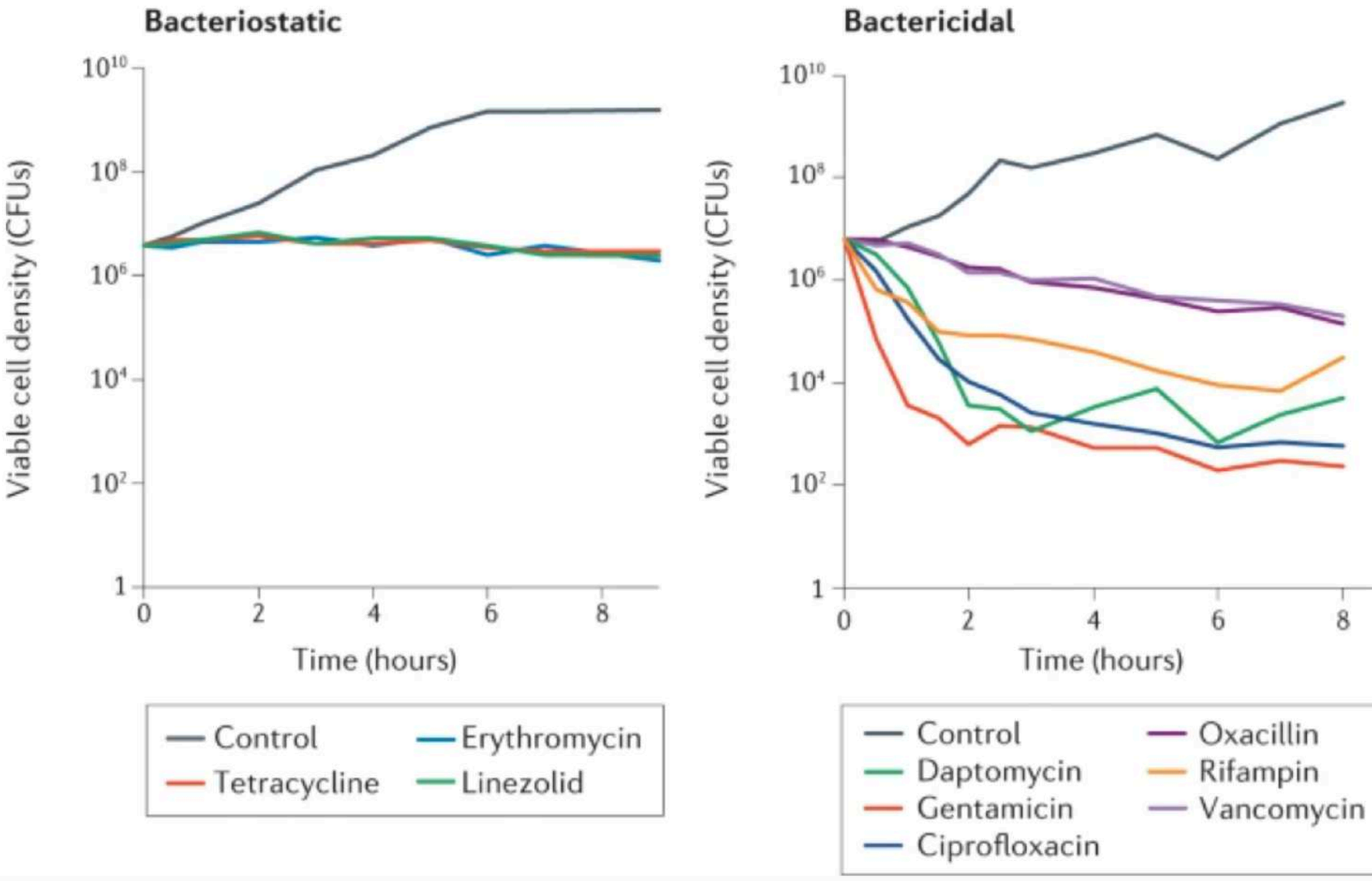
"Bacteriostatic" means that the agent prevents the growth of bacteria (i.e., it keeps them in the stationary phase of growth)

"Bactericidal" means that it kills bacteria, not by inhibiting the targets, but by corrupting them, leading to toxic products

- The most common measure of the level of resistance is the minimum inhibitory concentration (MIC), which is the lowest concentration of the antibiotic required to prevent the replication of the bacteria
- A higher MIC corresponds with a higher level of resistance

Antibiotic:
Bacteriostatic *vs.* Bactericidal, II

Fig. 1: Killing rates of different antibiotics.



Baquero and Levin, 2020

Antibiotic treatment failure is a substantial problem in modern medicine

Resistance, heteroresistance, tolerance and persistence

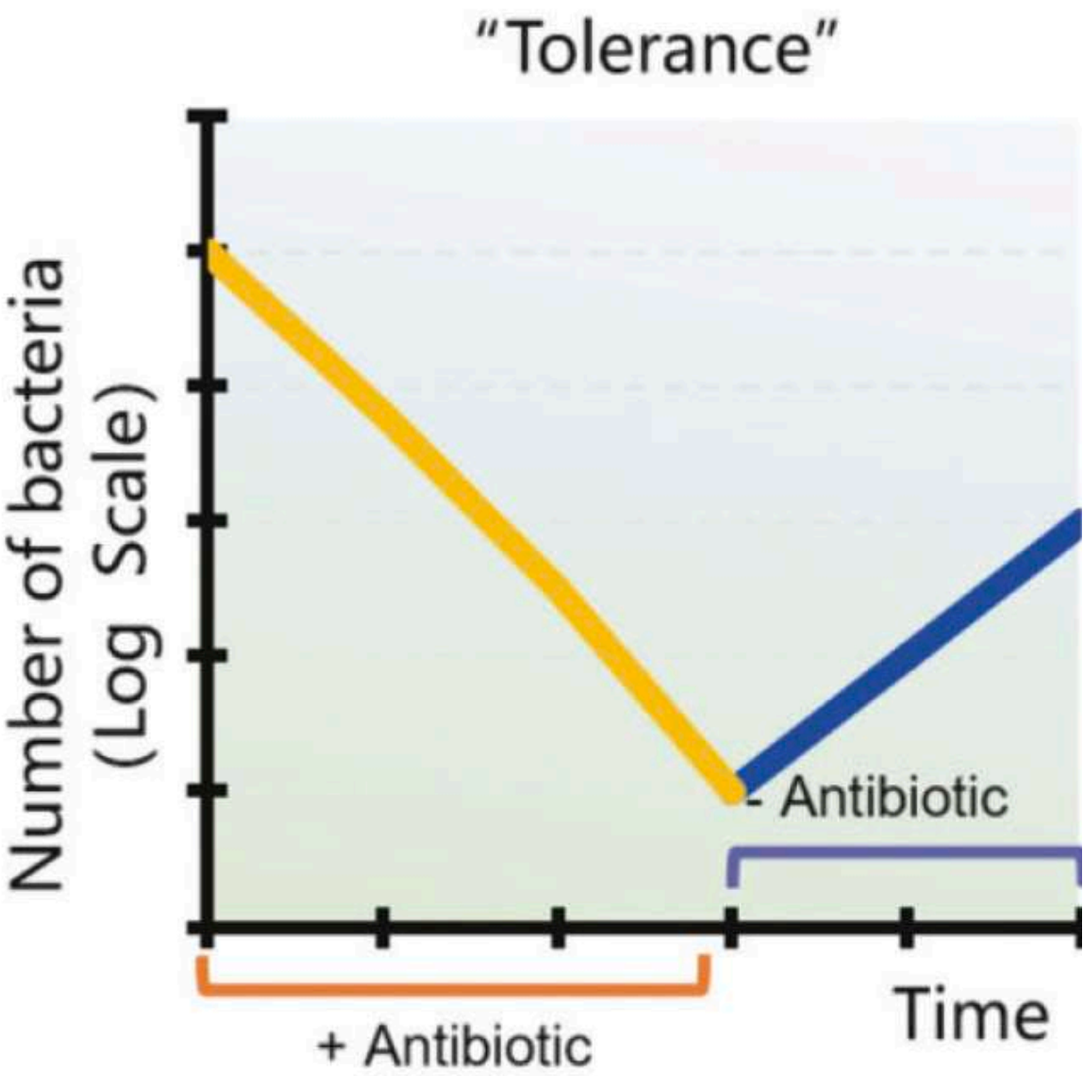
Resistance
The genetically encoded ability of cells to grow in the presence of an antibiotic. Resistance increases the minimum inhibitory concentration of an antibiotic compared with susceptible cells. The offspring remains resistant, even if grown in the absence of antibiotics.

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.

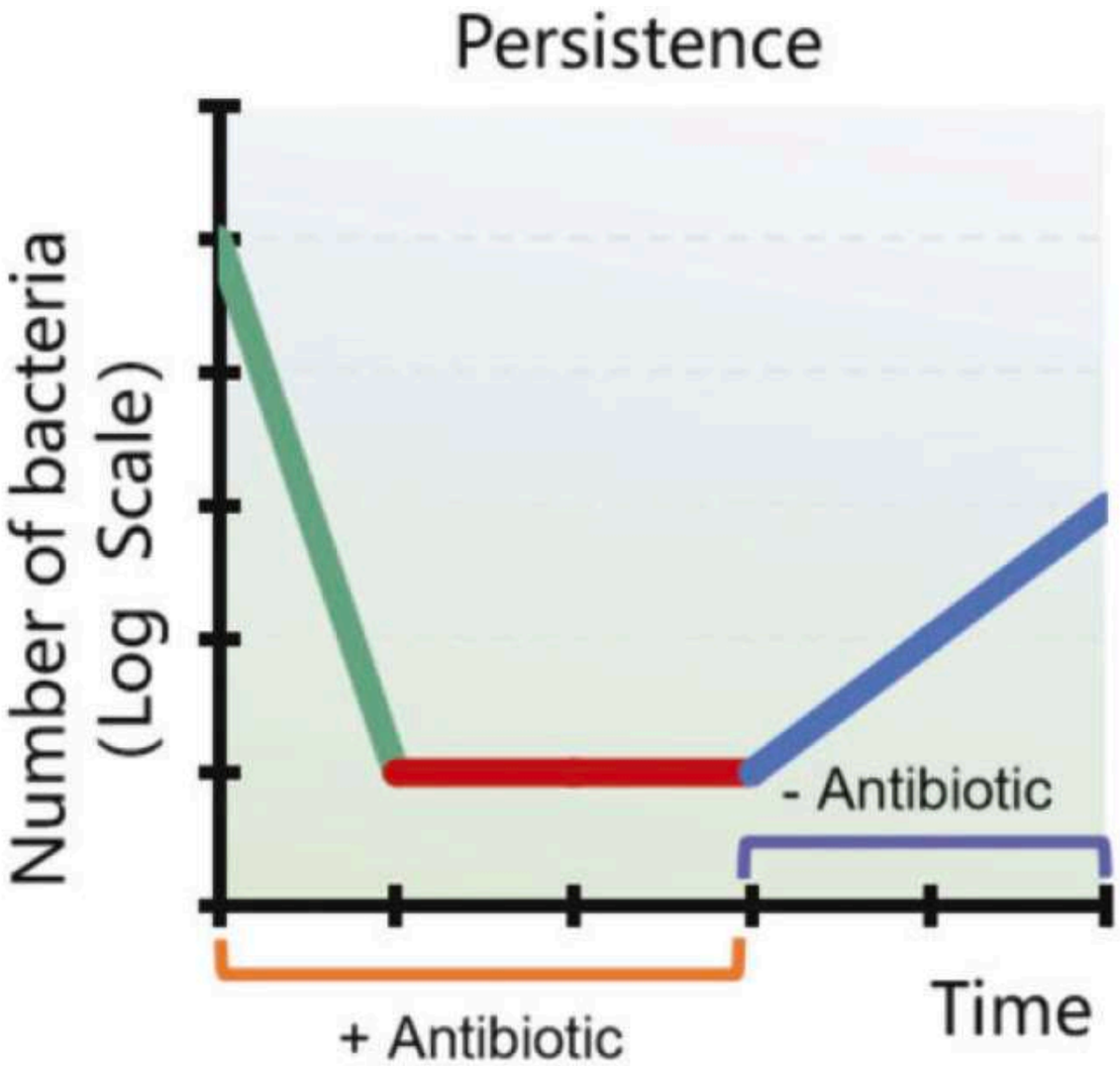
Persistence
The phenomenon that for a population in which two or more distinct subpopulations exist (susceptible and tolerant), treatment with a bactericidal antibiotic will kill the susceptible subpopulation quickly, simultaneous with a much slower killing of the tolerant subpopulation. This leads to 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

Tolerance



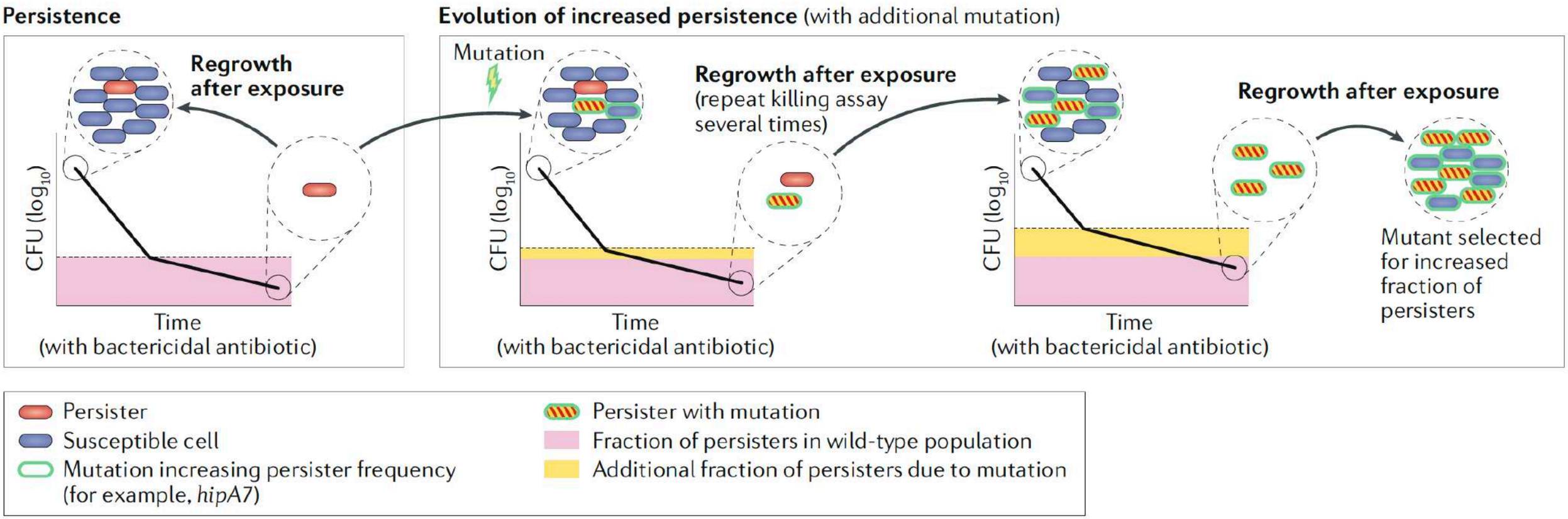
Persistence



Niu et al., 2024

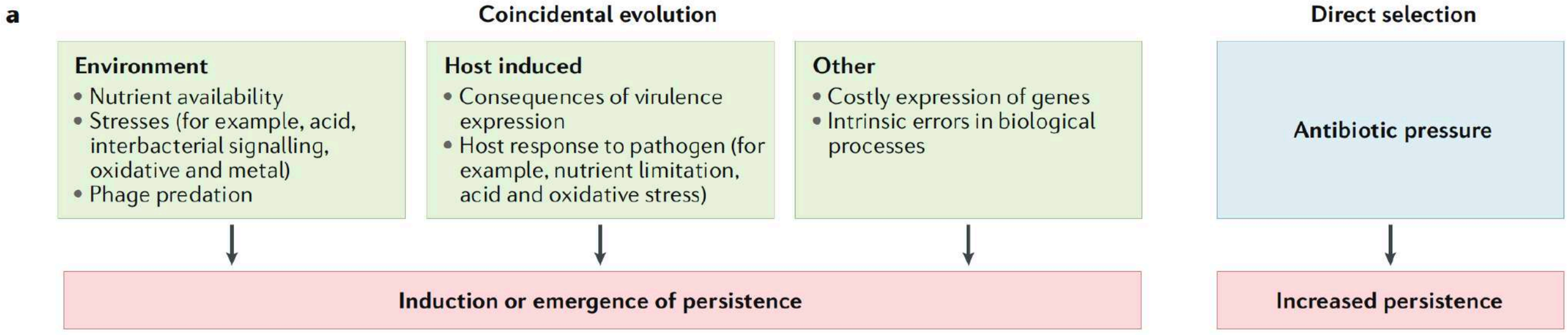
- Bacterial persister cells represent a subpopulation of cells that can survive intensive antibiotic treatment without being resistant
- Biphasic curve: not all bacteria in a clonal culture are killed at the same rate, no increase in the MIC
- Progeny is susceptible to antibiotic

Persistence dynamics



- Persister bacteria cannot replicate in the presence of the drug any better than the non- persister cells but are killed at a lower rate than the susceptible population from which they arose
- dormancy, reduced metabolism and ATP levels

Bakkeren et al., 2020



Bakkeren et al., 2020

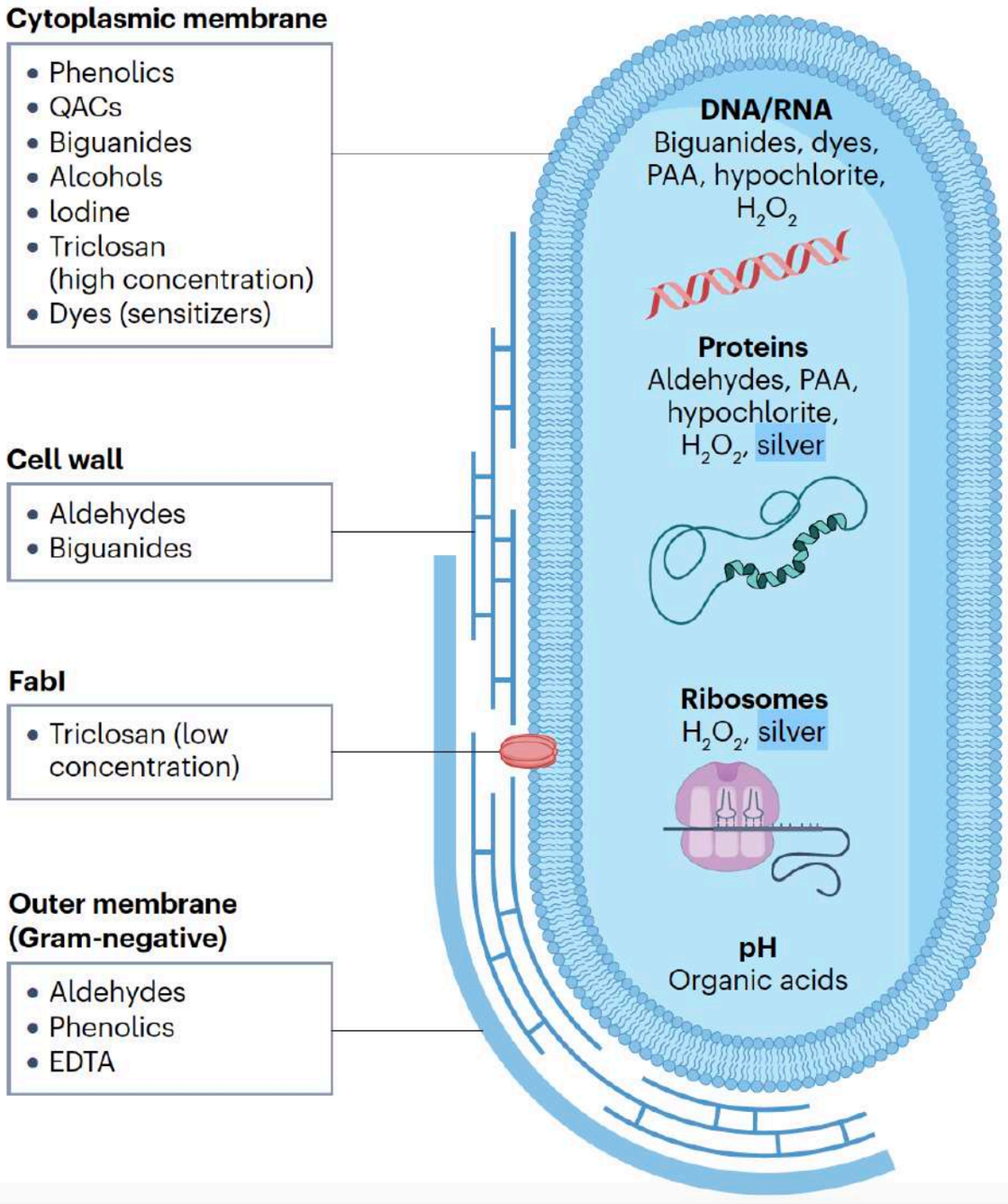
Genes and pathways involved in persister formation or survival

Pathways	
Toxin-antitoxin module	
Metabolism	Energy metabolism
	Protein degradation systems and trans-translation
	Purine and amino acid metabolism
	Metabolic regulators
DNA damage repair	
Stress response	SOS response
	RpoS (σS) -mediated response
Cellular communication	Quorum sensing molecules
	Stringent response and ppGpp
Efflux pump system	
Epigenetic modifications and others	

Niu et al., 2024

Disinfectants and antiseptics

Generalmente il termine disinfettante indica un prodotto da utilizzare su oggetti inanimati, il termine antisettico indica un prodotto da utilizzare sui tessuti vivent



Maillard and Pascoe, 2024

Table 1 | Major types of biocides and their mechanisms of action

Types	Mechanism of action	Examples of chemistry	Application and areas of use
Highly reactive biocides — strong interactions through chemical or ionic binding			
Alkylating agents	Reacts with amino acids to form crosslinks and fix proteins	Glutaraldehyde, formaldehyde, ortho-phthalaldehyde	Disinfection of surfaces, materials, equipment Disinfection of materials and surfaces associated with the housing or transportation of animals
Oxidizing agents	Oxidation of macromolecules (proteins, lipids and nucleotides), while causing nonspecific damage to the cytoplasmic membrane	Sodium hypochlorite, peracetic acid, hydrogen peroxide, ethylene oxide	Disinfection of surfaces, materials, equipment Disinfection of materials and surfaces associated with the housing or transportation of animals Disinfection of drinking water
		Povidone–iodine	Disinfection of skin, scalps, surfaces, materials and equipment
Less-reactive biocides — weak physical interaction			
Cationics	Positively charged, hydrophilic region interacts with negatively charged cell surface. Hydrophobic region partitions into membrane, disrupting intermolecular bonds and leading to loss of intracellular contents	Quaternary ammonium compounds (for example, benzalkonium chloride)	Disinfection of skin and scalps Disinfection of surfaces, materials and equipment Incorporated in textiles, tissues, mask, producing treated articles with self-disinfecting properties
		Biguanides (for example, chlorhexidine, polyhexamethylene biguanide)	Antisepsis of skin and scalps Disinfection of surfaces, materials, equipment and swimming pools
		Diamines and amine oxides	Disinfection of surfaces, materials and equipment

Maillard and Pascoe, 2024

Phenolics	Protonophore that targets the cytoplasmic membrane, causing loss of membrane potential. At low concentrations, triclosan inhibits fatty acid synthesis	Triclosan	Disinfection of surfaces, materials and equipment Incorporated in textiles, tissues, mask, producing treated articles with disinfecting properties
Alcohols	Permeabilization of the cytoplasmic membrane, denaturation of proteins and dehydration of exposed bacteria	Ethyl alcohol (ethanol) and isopropyl alcohol	Disinfection of skin and scalps Disinfection of surfaces, materials and equipment
Weak organic acids	Uncoupling of proton motive force; acidification of bacterial cytoplasm, leading to inhibition of enzyme activity and biosynthesis while exerting osmotic stress	Citric acid and benzoic acid	Disinfection of skin and scalps Disinfection of surfaces, materials and equipment
Metal ions	Redox active. Interacts with thiol groups and generates reactive oxygen species that damage macromolecules	Silver and copper	Antimicrobial surfaces, textiles and wound dressings
Antimicrobial dyes	Intercalation with DNA. Production of singlet oxygen (photosensitizers)	Methylene blue, toluidine blue and crystal violet	Wound dressings, photodynamic therapy (photosensitizers)

Information based partly on refs. 21,27.

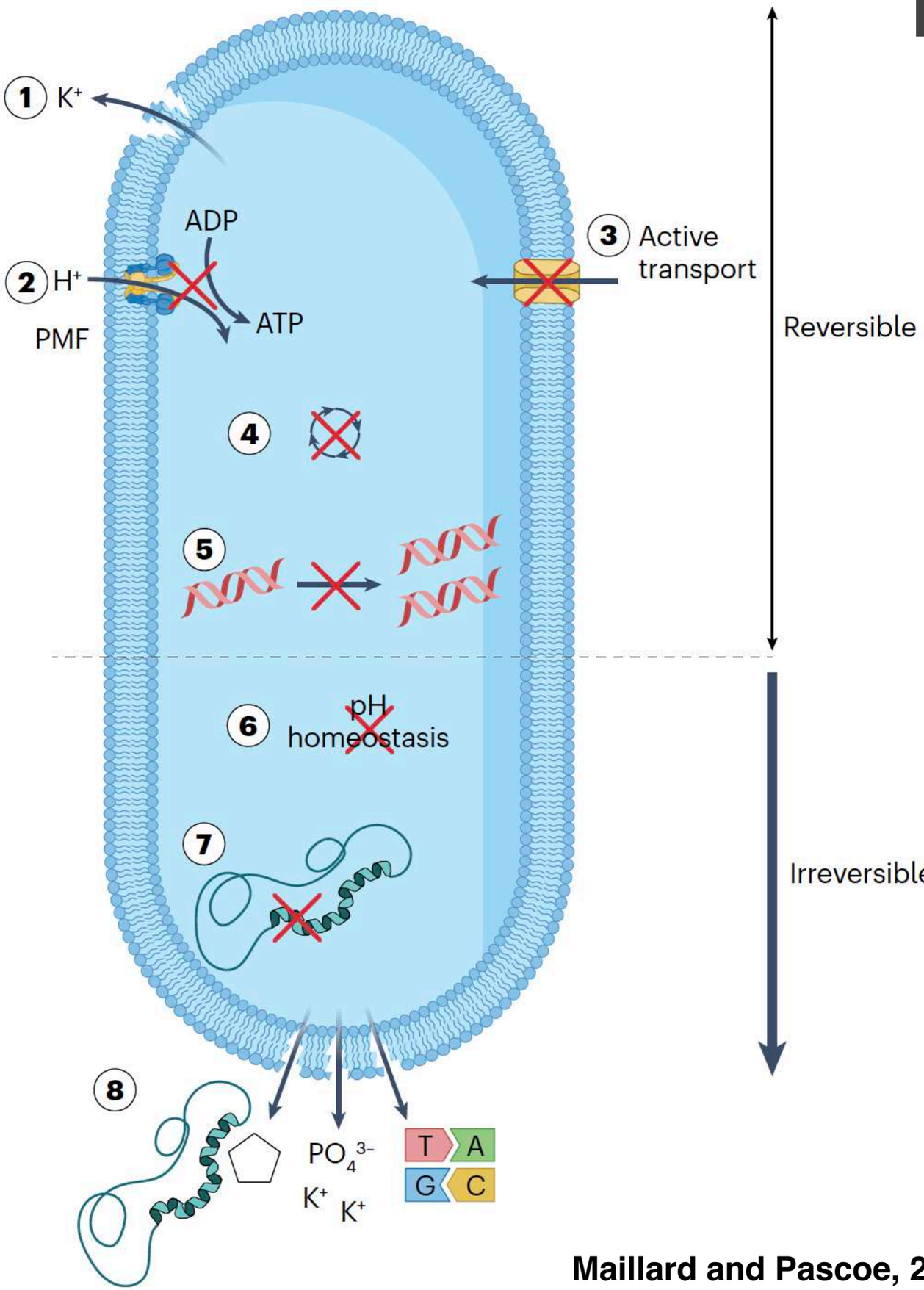
Maillard and Pascoe, 2024

Table 2 | Extrinsic factors affecting the performance of biocides

Factors		Comments
Biocide properties	Mechanism of action	Spectrum of activity determined by chemistry underlying biocide–microorganism interaction
	Use concentration	Concentration correlates with speed of effect
	Formulation and product composition	Excipients, co-actives and pH may affect biocide reactivity, interaction with bacterial cells (for example, EDTA destabilization of the outer membrane), drying time (formulation to wipe ratio) and surface wettability (surfactants)
Application factors	Contact time	Level of inactivation partially determined by time (disinfection kinetic)
	Presence of organic soils (has the surface been cleaned?)	Organic matter may react with biocides and reduce performance
	Surface type	Performance may be affected by the target surface (for example, polyvinyl chloride (PVC) versus stainless steel)
	Environmental temperature	Increased temperature increases rate of reaction
	Method of delivery (for example, vaporization, spraying, wiping)	Efficacy of a biocide will change if it is in a liquid or gas form. The method of delivery will also impact on the overall efficacy of the formulation
	Interactions between biocide and applicator	Some biocides may interact with applicator (for example, wipe material), reducing effective concentration
	Concentration on subsequent dilution and abrasion	Reduction in concentration during use may reduce biocidal efficacy
Target organism	Endospores	Metabolically inactive structures of *Bacillus* spp. and *Clostridioides* spp. highly tolerate biocide exposure (Fig. 3)
	Bacterial type (for example, mycobacteria and Gram-negative species)	Intrinsic factors may affect resistance to specific biocides (for example, outer membrane and quaternary ammonium compounds)
	Metabolic activity	Reduced metabolism associated with decreased susceptibility
	Lifestyle (Supplementary Box 1)	Microbial communities (biofilms) exhibit reduced susceptibility to antimicrobials

Maillard and Pascoe, 2024

Mechanisms of action



Reversible events: **release** of intracellular **potassium** (1), which causes a **depletion of the membrane potential** and loss of proton motive force (PMF) necessary for ATP biosynthesis (2)

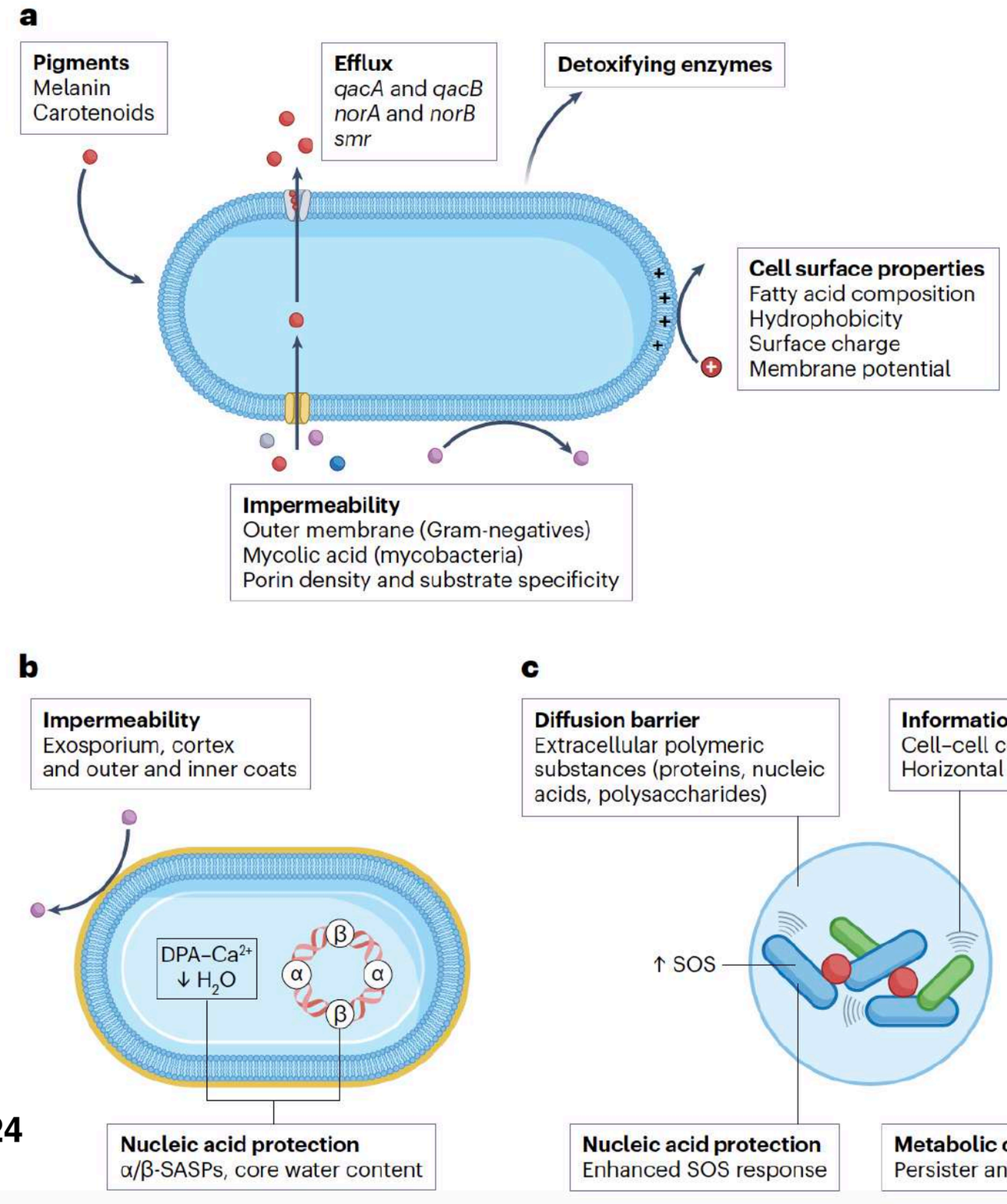
This leads to an **arrest of active transport (3), normal metabolic processes (4) and replication (5)**

Irreversible damage: c**hanges to cytosolic pH** (6), which cascades into **disruption of enzymatic function and coagulation of intracellular material** (7). If the **cytoplasmic membrane** becomes significantly **damaged**, cytoplasmic constituents including proteins, nucleotides, pentoses and other ions may be lost from the cell (8)

EDTA disrupts the outer membrane of Gram-negative bacteria, potentiating biocidal effects
H2O2, hydrogen peroxide; K+, potassium ion; PAA, peracetic acid; PO4 3–, phosphate; QACs, quaternary ammonium compounds

Maillard and Pascoe, 2024

Microbial response to disinfectants and antiseptics



Maillard and Pascoe, 2024