Qazvin University of Medical Sciences

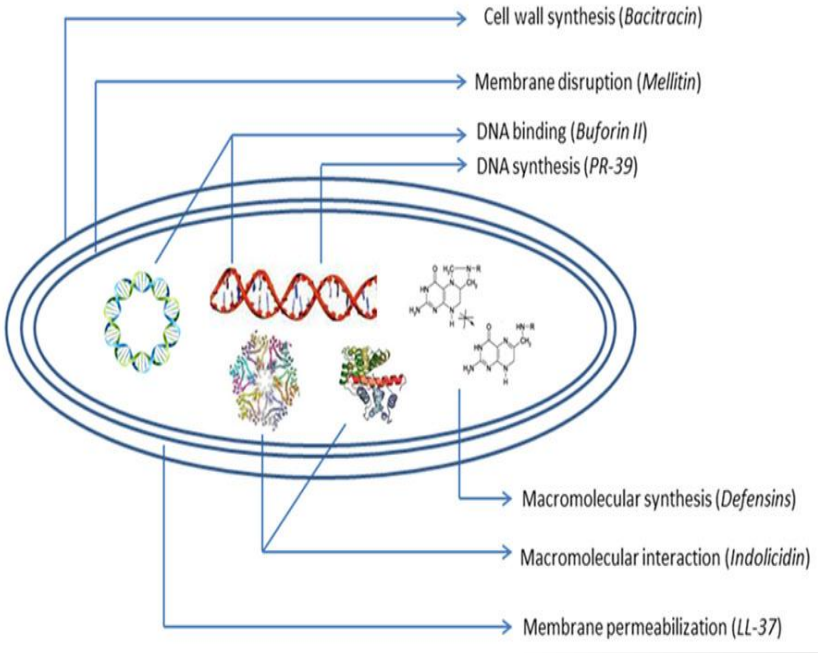
Antimicrobial Peptide

PRESENTED BY:

Ziba Galili

Supervised by :

Dr. M. Aslanimehr



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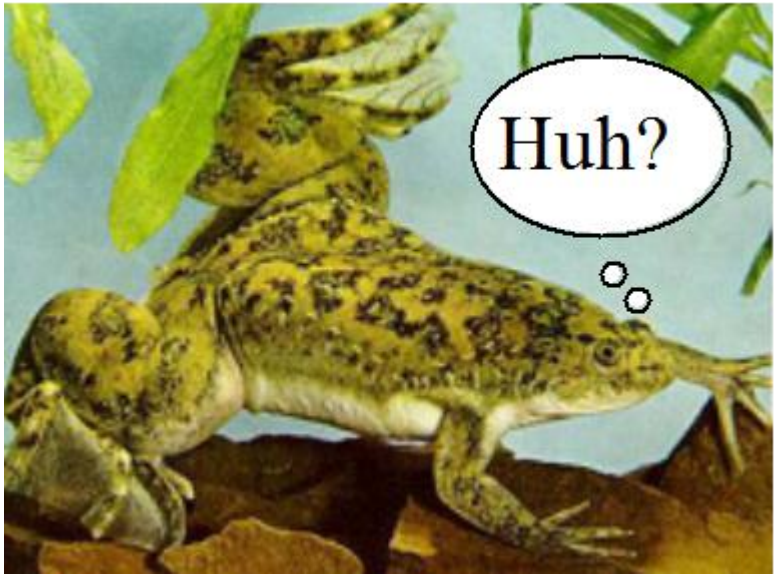
This frog could save your leg



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Antimicrobial peptides: *Where are they?*

- ***In everything from Amoebas to Humans***
- ***Abundant in vertebrates in:***
  - ***External Mucosa***
    - ***eyes, mouth, genitourinary, skin, lung, trachea***
  - ***Immune cells***
    - ***neutrophils***
  - ***Intestinal tract (duodenum)***
    - ***in humans; Paneth cells are the source***

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Antimicrobial peptides: What are they?

- Antimicrobial peptides (AMPs) are natural antibiotics produced by various organisms such as mammals, arthropods, plants, and bacteria

- In addition to antimicrobial activity, AMPs can induce chemokine production, accelerate angiogenesis, and wound healing and modulate apoptosis in multicellular organisms.

- currently,more than 2000 AMPs have been reported in antimicrobial peptide database(http://aps.unmc.edu/AP/main.php/) .

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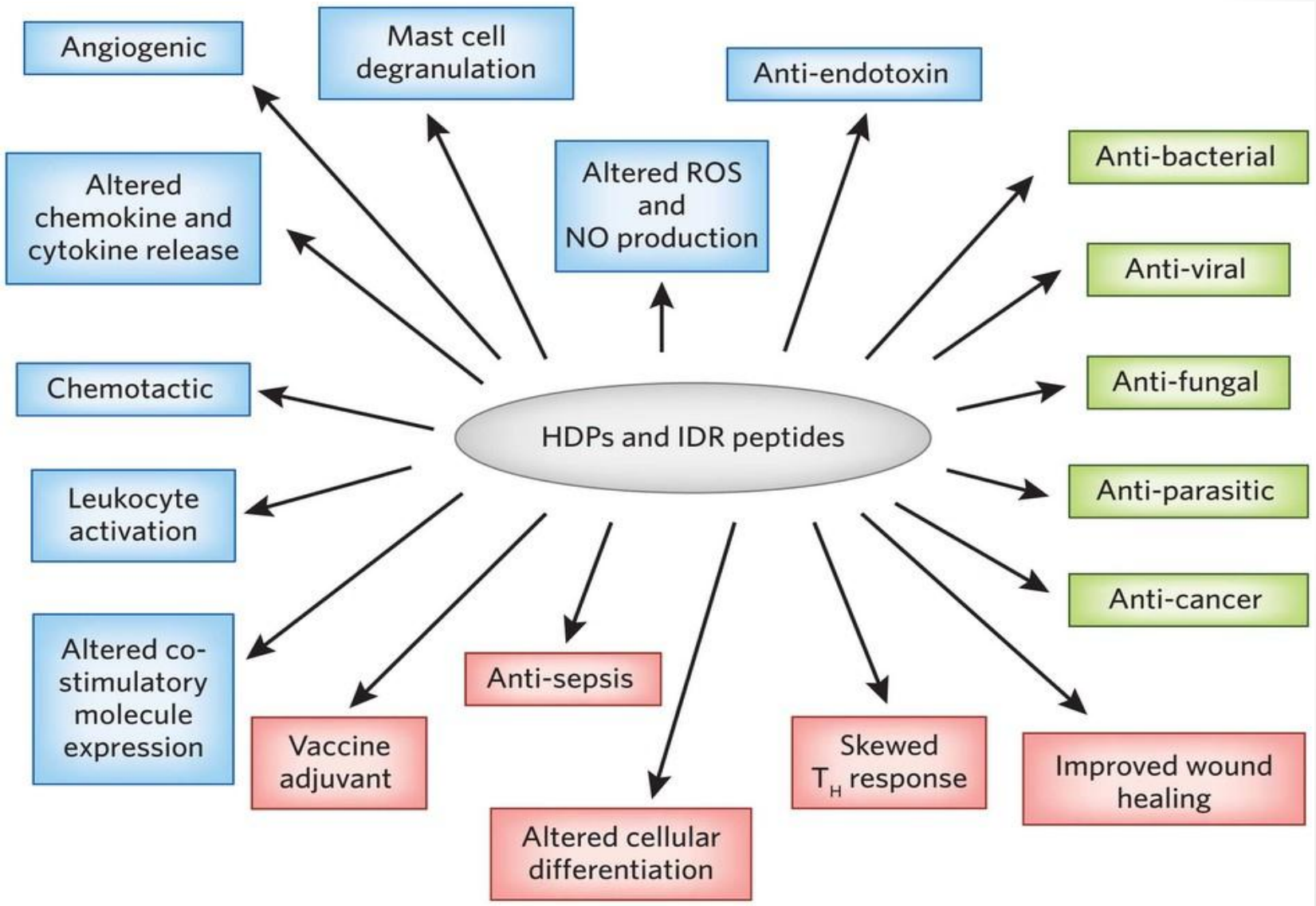

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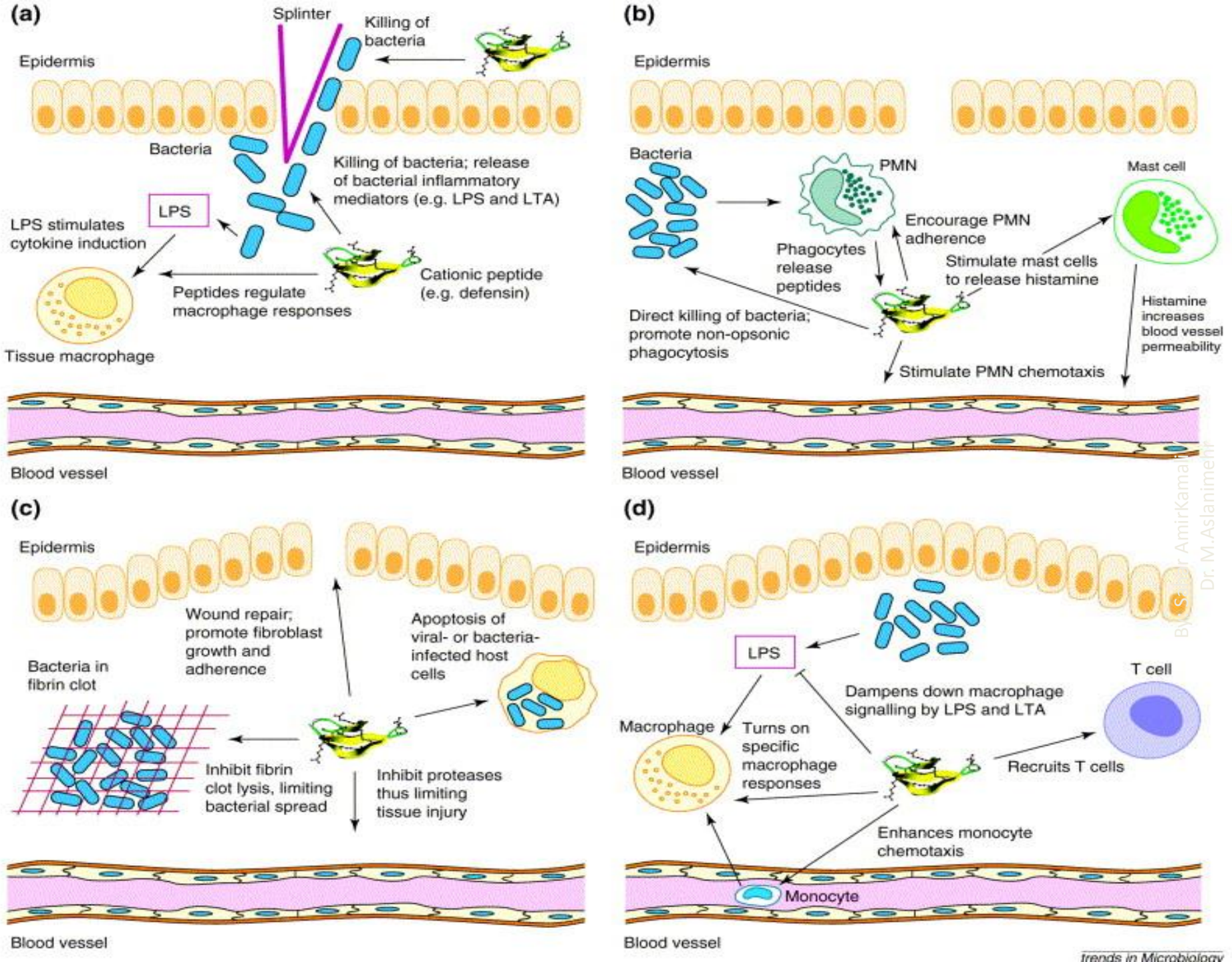
(a)
Splinter
Killing of bacteria
Epidermis
Bacteria
Killing of bacteria; release of bacterial inflammatory mediators (e.g. LPS and LTA)
LPS
LPS stimulates cytokine induction
Cationic peptide (e.g. defensin)
Peptides regulate macrophage responses
Tissue macrophage
Blood vessel
(b)
Epidermis
Bacteria
PMN
Mast cell
Encourage PMN adherence
Phagocytes release peptides
Stimulate mast cells to release histamine
Histamine increases blood vessel permeability
Direct killing of bacteria; promote non-opsonic phagocytosis
Stimulate PMN chemotaxis
Blood vessel
(c)
Epidermis
Wound repair; promote fibroblast growth and adherence
Apoptosis of viral- or bacteria-infected host cells
Bacteria in fibrin clot
Inhibit fibrin clot lysis, limiting bacterial spread
Inhibit proteases thus limiting tissue injury
Blood vessel
(d)
Epidermis
LPS
T cell
Dampens down macrophage signalling by LPS and LTA
Macrophage
Turns on specific macrophage responses
Recruits T cells
Enhances monocyte chemotaxis
Monocyte
Blood vessel
trends in Microbiology

Classification of Antimicrobial Peptides

There are numerous ways for classifying antimicrobial peptides.

1. Based on the biosynthetic machine

Natural peptides can be classified as gene coded and non-gene coded (i.e. multiple enzyme systems).

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2. Based on biological source

- Bacterial AMPs (bacteriocins)
- plant AMPs
- animal AMPs.

Animal AMPs are further classified into insect AMPs, amphibian AMPs, fish AMPs, reptile AMPs, mammal AMPs, etc based on source family names.

- The major and well-studied AMPs families in the animal kingdom are cathelicidins, defensins, and histatins.

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Various sources of AMPs

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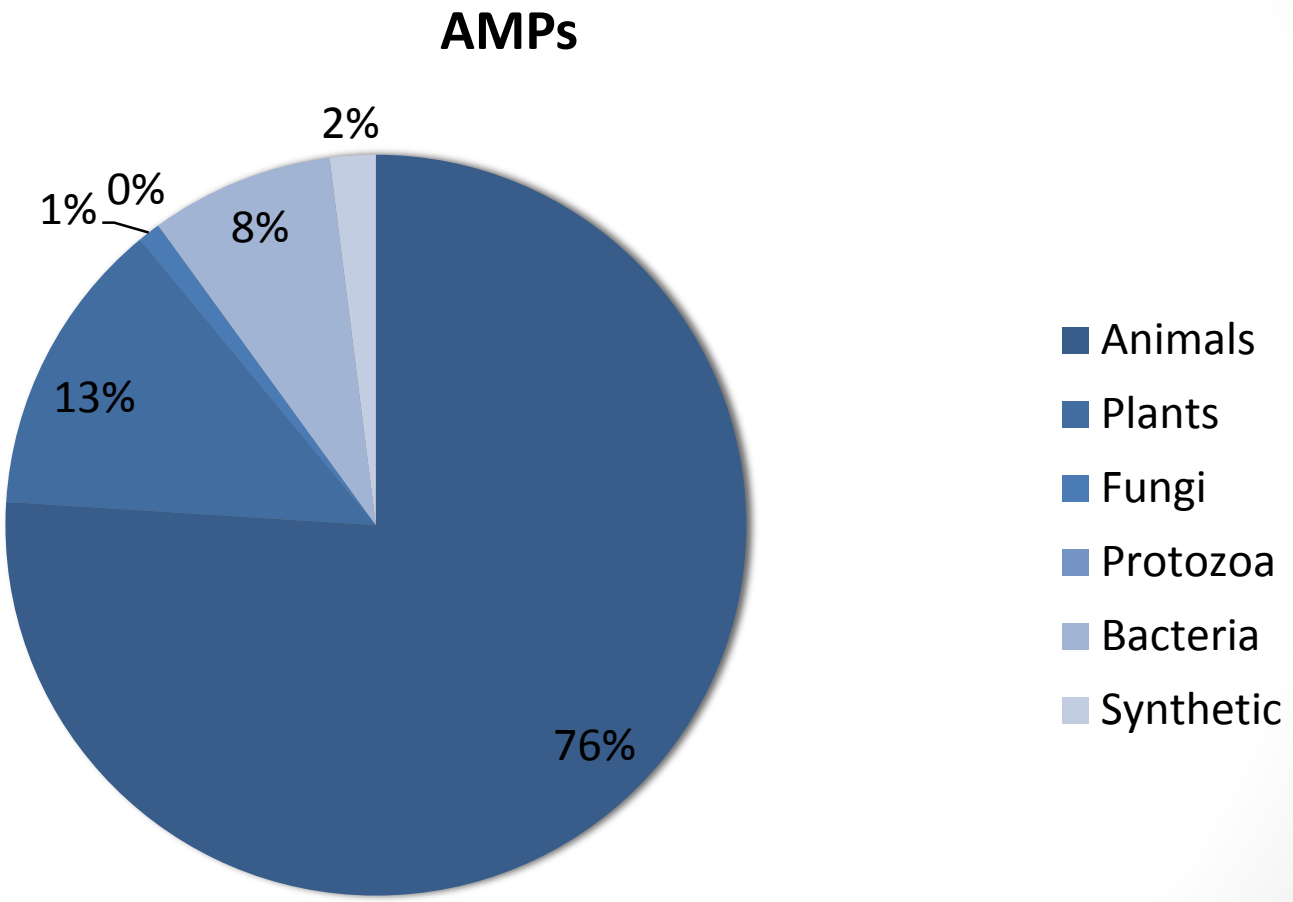
Source of AMPs	AMPs	REFERENCES
insect	cecropinA,SarotoxinIA,PonericinG2,Ceratotoxin,stomoxyn,spinigerenin,thanatin,heliomicin,Alo3,sapecin,defensinA,smD1,gallerimycin,termicin,royalisin,drosomycin,drosocin,metchnikowin,apidaecinIA,abaecin,formaecin,lebocin,pyrrhocoricin,melittin,attacins,coleoptericin,diptericin,	[9,10]
Fishes	Pardaxins,misgurin,pleurocidins,parasin,oncorhyncin II and III ,chrysophsin and HFIAP	[18[
Amphibians	Japonicin-1&2,nigrocin1&2,brevinin-20a,temporin-1Od,tigerin-1,pseudin-2,maximin-1,distinctin	[11]
Echinoderms	Strongylocins,centrocins,betathymosins.filaminA	[12]
Crustaceans	Callinectin,astacidin2,armadillidin,homarin,scygonadin,penaeidin,crrustin.hyastatin,arasin,stylicin,hemocyanin derived peptides	[13]
Plants	Thionins,plant defensins,lipid transfer protein	[14]
Mammals	Defensin,histatin,LL-37,indolicidin,protegrin,lactoferricin	[15]
Bacteria	Iturin,bacillomycin,syringomycin,syringostatins,syringotoxins,nikkomycins	[16]
Fungi	Echinocandins,aculeacins,mulundocandins,FK463,aureobasidin, leucinostatins,helioferins	[17]

Various sources of AMPs



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5. Based on covalent bonding pattern

- This universal classification system (UC) led to four classes of antimicrobial peptides (Wang, G, 2015, see ref. below).
- (1) UCLL: linear one chain peptides (e.g. LL-37 and magainins) or peptides with two unlinked chains (e.g. enterocin L50).
- (2) UCSS: sidechain-linked peptides (e.g. disulfide-containing defensins or ether bond-containing lantibiotics). A sidechain-sidechain connection can occur within a single peptide chain or between two different peptide chains.
- (3) UCSB: polypeptide chains with a sidechain to backbone connection (e.g. bacterial lassos and fusaricidins).
- (4) UCBB: the formation of a peptide bond between the N- and C-termini. Circular peptides have been found in bacteria (e.g. AS-48), plants (e.g. cyclotides), and animals (e.g. theta-defensins).

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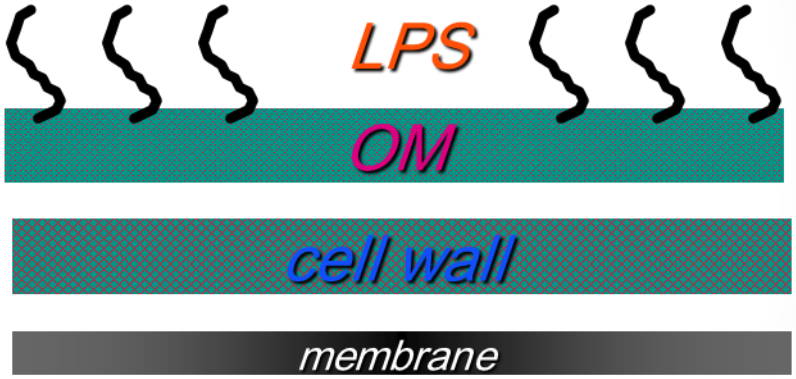
3. Based on biological functions

- ✓ Antibacterial
  - Gram positive

  - Gram negative



- ✓ antiviral
- ✓ antifungal
- ✓ antiparasital
- ✓ insecticidal
- ✓ chemotactic
- ✓ wound healing
- ✓ growth promotion, etc.

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4. Based on molecular properties

- In the absence of three-dimensional (3D) structural information, AMPs can be classified based on peptide properties such as charge, hydrophobicity, length, motif, etc. For example,

- there are cationic, neutral, and anionic peptides based on net charge. (Although the antibacterial activity of anionic peptides is considered weak, they could improve the activity of cationic peptides)

- There are hydrophobic, amphipathic, and hydrophilic peptides based on hydrophobicity.

- Also, natural AMPs can be classified based on peptide size: ultra-small (2-10 aa), small (10-24 aa), medium (25-50 aa), and large (50-100 aa). AMPs greater than 100 aa are antimicrobial proteins.

Antimicrobial peptides:
The largest group corresponds to cationic peptides what are they?

❖Cationic, Amphipathic

- Linear alpha-helical (20-35 AA)

- Magainin, cecropins, histatin

- Pro-Arg rich (more polar)

- bac 5 and 7 (30-50 AA; 70% pro/arg)

- Disulfide Rich alpha-structure

- amoebapores, NK-lysin

- Disulfide Rich beta-structure

- α-, β-, and θ Defensins: three or more disulfide (30-70 AA)

- "Loops" (11-22 AA)

tachpyylesins, protegrins ,ranalexin, polymyxin

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6. Based on 3D structure

AMPs are classified into four families:

- alpha(consists of AMPs with helical structures (e.g. magainins and LL-37)
- beta(AMPs with beta-strands (e.g. human alpha-defensins).
- alphabeta(comprises both helical and beta-strands in the 3D structure (e.g. beta-defensins)
- non-alphabeta(contains neither helical nor beta-strands (e.g. indolicidin).

They are:

(1)frog magainin 2, alpha-helical

(2) lactoferricin B, beta-sheet

(3) plant defensin Psd1, alpha-beta structure

(4) bovine indolicidin, non-alpha-beta structure

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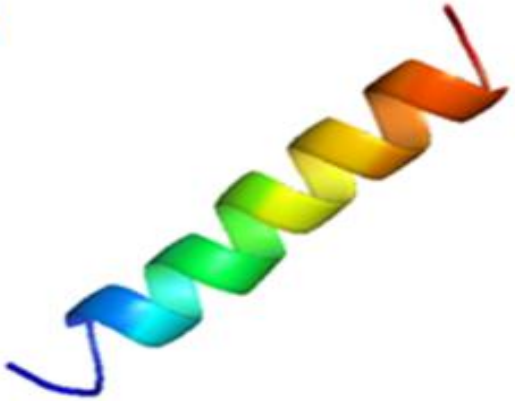
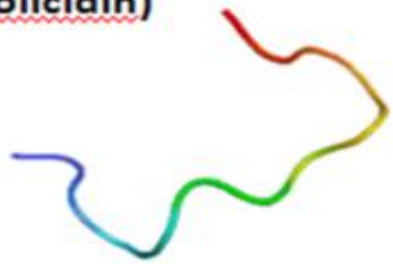
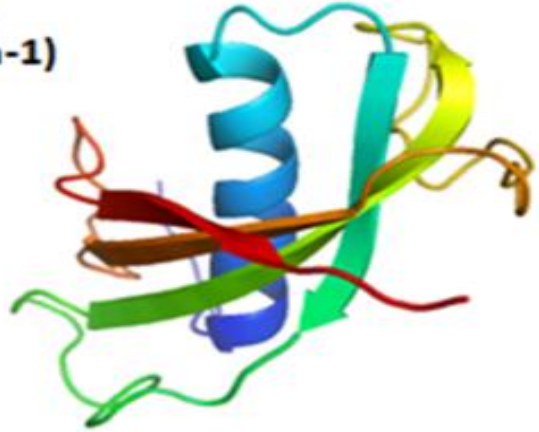
α-helical
(Magainin)



Extended
(Indolicidin)



Mixed
(Protegrin-1)



B-sheet
(defensin.human)

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List of antimicrobial peptides based on their structural features.

Class of AMP	Structural features	Representative peptides	structure	References
Cationic peptides	-Peptides forming α – helical structures	Cecropins	α-Helix	[19]
	-Single disulphide bridge	Thanatin	β-Sheet	[20]
	-Tow disulphide bridge	Tachyplesin II	β-Sheet	[21]
	-Three disulphide bridge	Penaeidins	β-Sheet	[22]
	-More than three disulphide bridge	Drosomycin	αβStruture	[20]
	-Proline-rich peptides	Pyrrhocoricin	αβStruture	[23]
	-glycine-rich peptide	Diptericins	-	[24]
	-Histidine-rich peptide	Histatin	Rich in H	[25]
	-Tryptopphan-rich peptide	indolicidin	extended	[26]

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Class of AMP	Structural features	Representative peptides	structure	Reference
Noncationic peptides	-Neuropeptide derived molecules	Secretolytin	α-Helix	[27]
	-Aspartic acid rich peptides	Dermcidin	-	[28]
	-Aromatic dipeptides	N-alanyl-5-s-glutathionyl-3,4dihydroxy-phenylalanine and p-hydroxy cinnamaldehyde	-	[29]
	-Oxygen binding proteins	lactoferricin	B-turn	[30]

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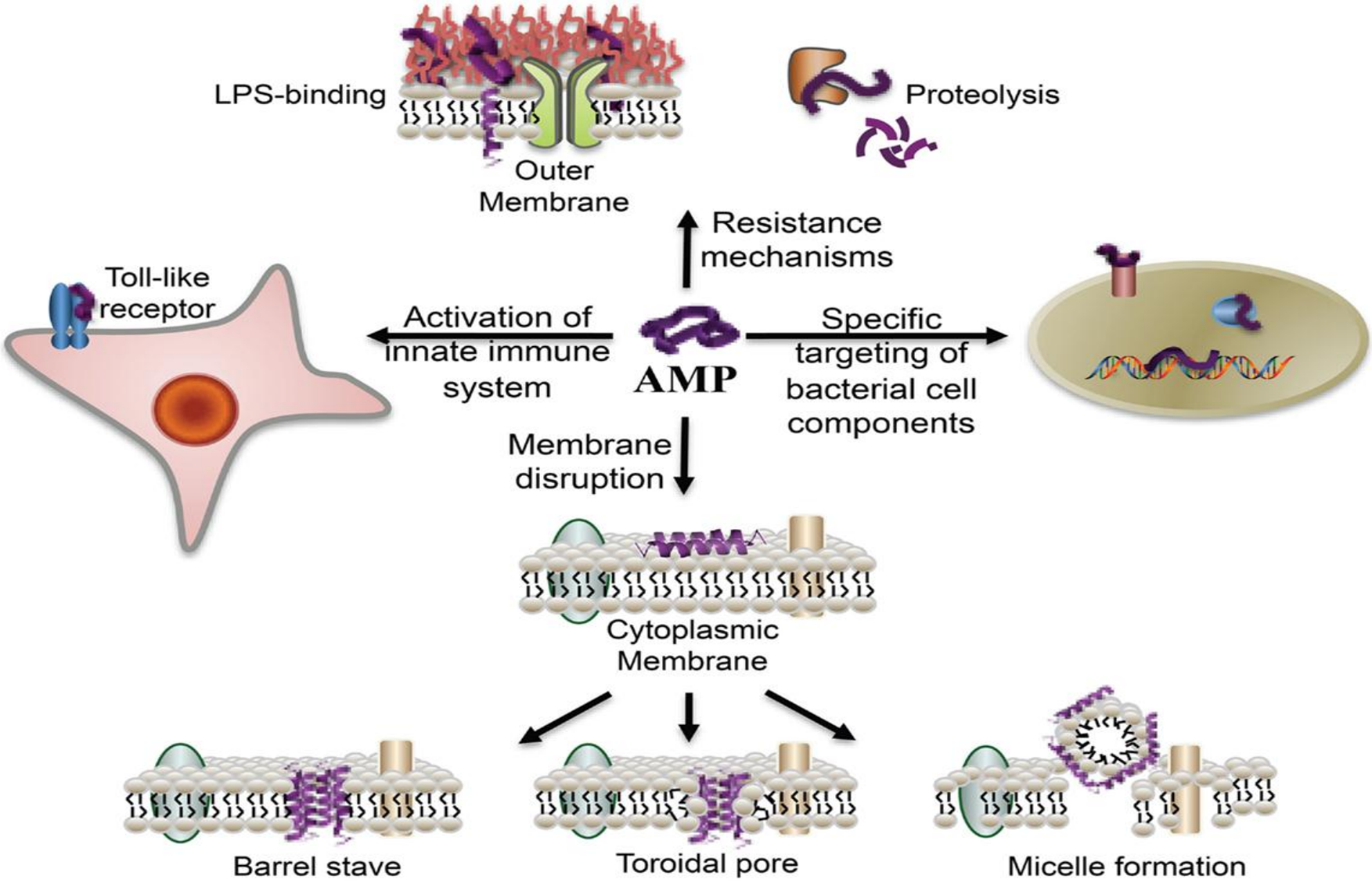
7. Based on molecular targets

AMPs can be broadly classified into two families:

- cell surface targeting peptides (e.g. nisins and temporins)
- intracellular targeting peptides (e.g. Pro-rich peptides).

Cell surface-targeting peptides, including both membrane-targeting and non-membrane targeting peptides, can be further classified based on specific targets such as cell wall/carbohydrates, lipids/membranes, and proteins/receptors.

intracellular targeting AMPs can be further classified based on the specific target molecules (e.g. heat shock proteins, DNA, and RNA).



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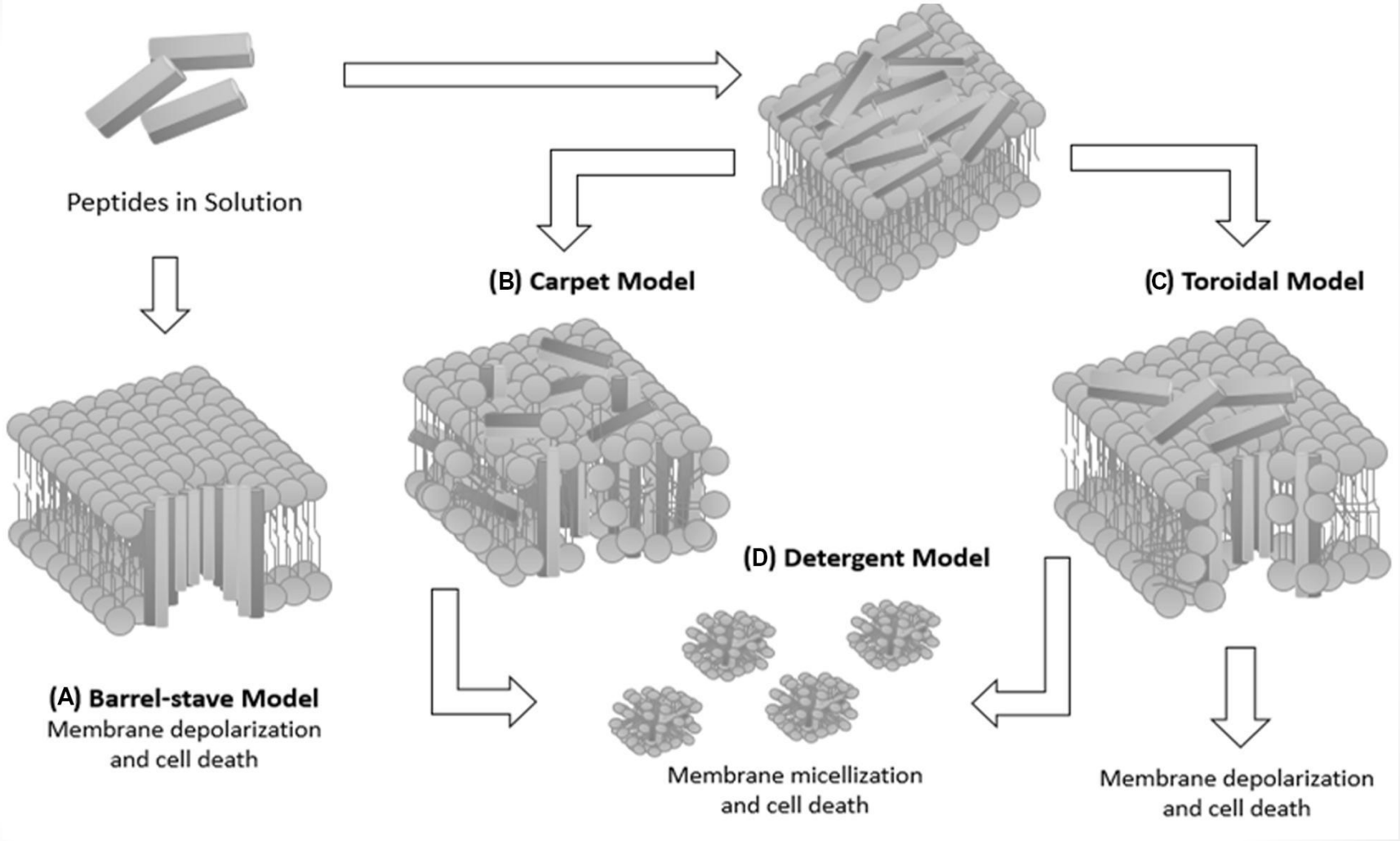

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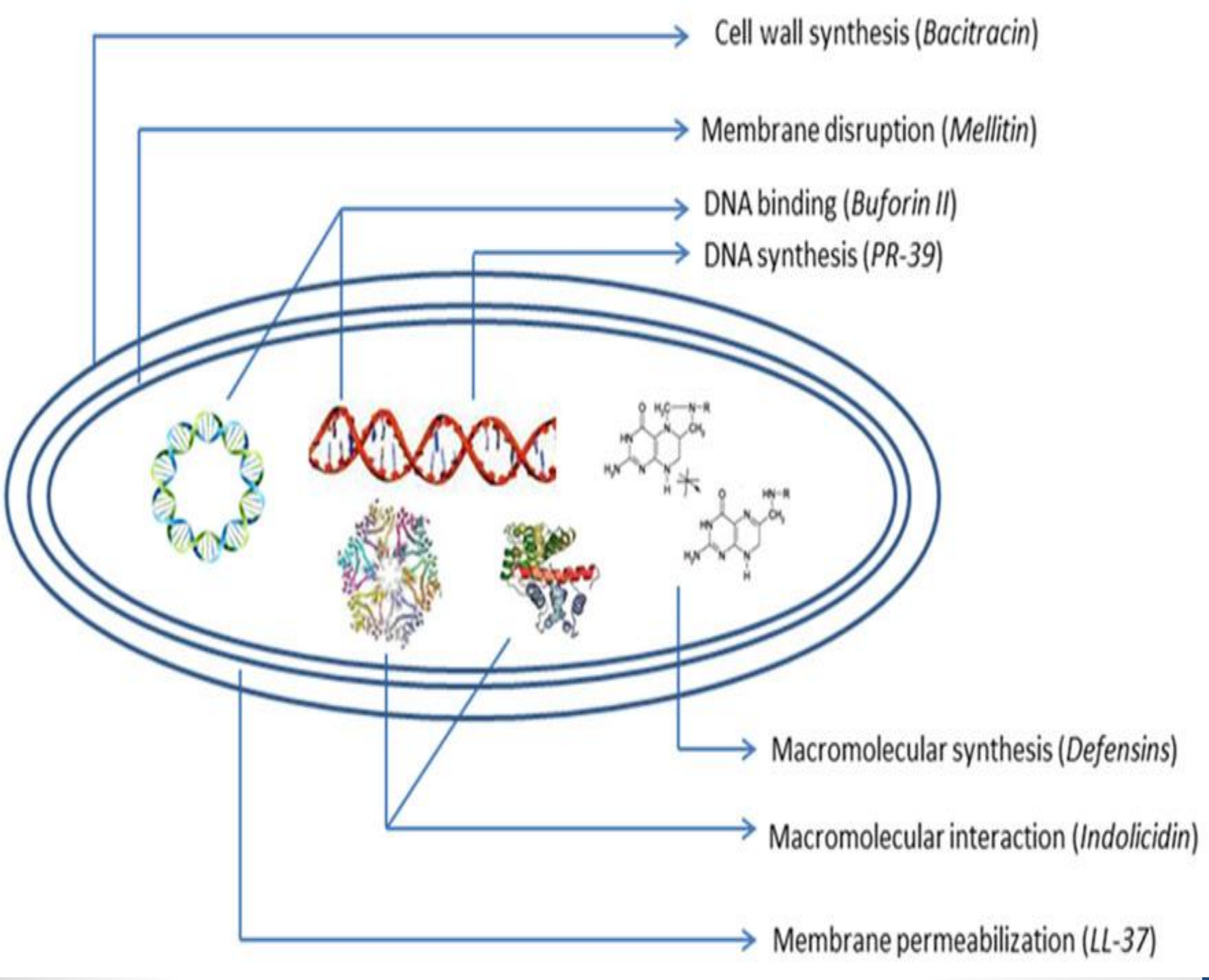

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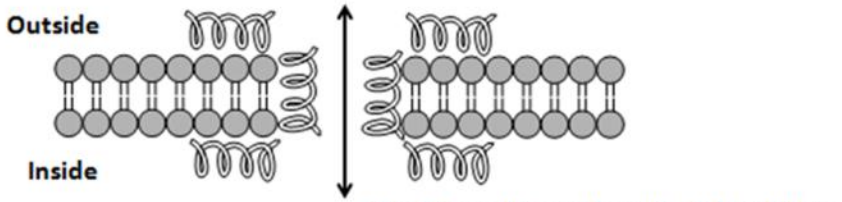
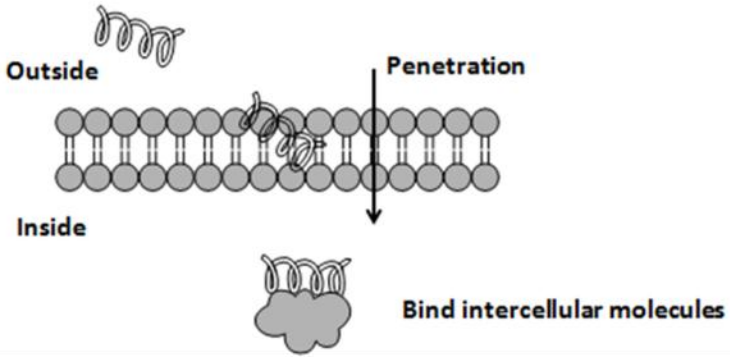
Transmembrane pore-forming



Modes of intracellular killing



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Antimicrobial peptides: Why are they picky?

Bacteria have :

- more negative charge
  - recall: peptides are cationic
- no sterols, little phosphatidylcholine or SM
- a lot of PG/PE/cardiolipin/PS
  - some tumor cells too!

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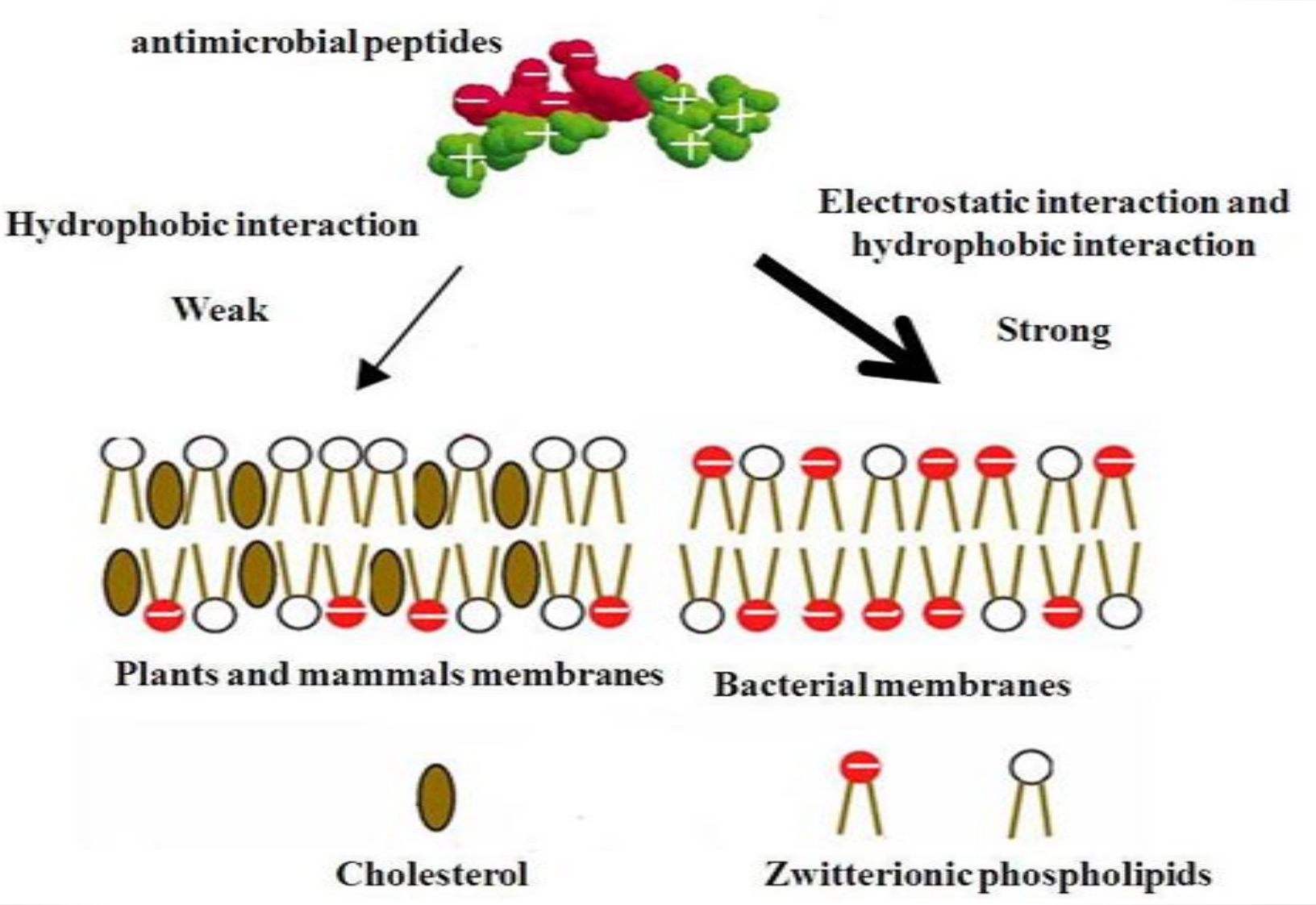
Difference between bacterial cell membrane and mammalian membranes



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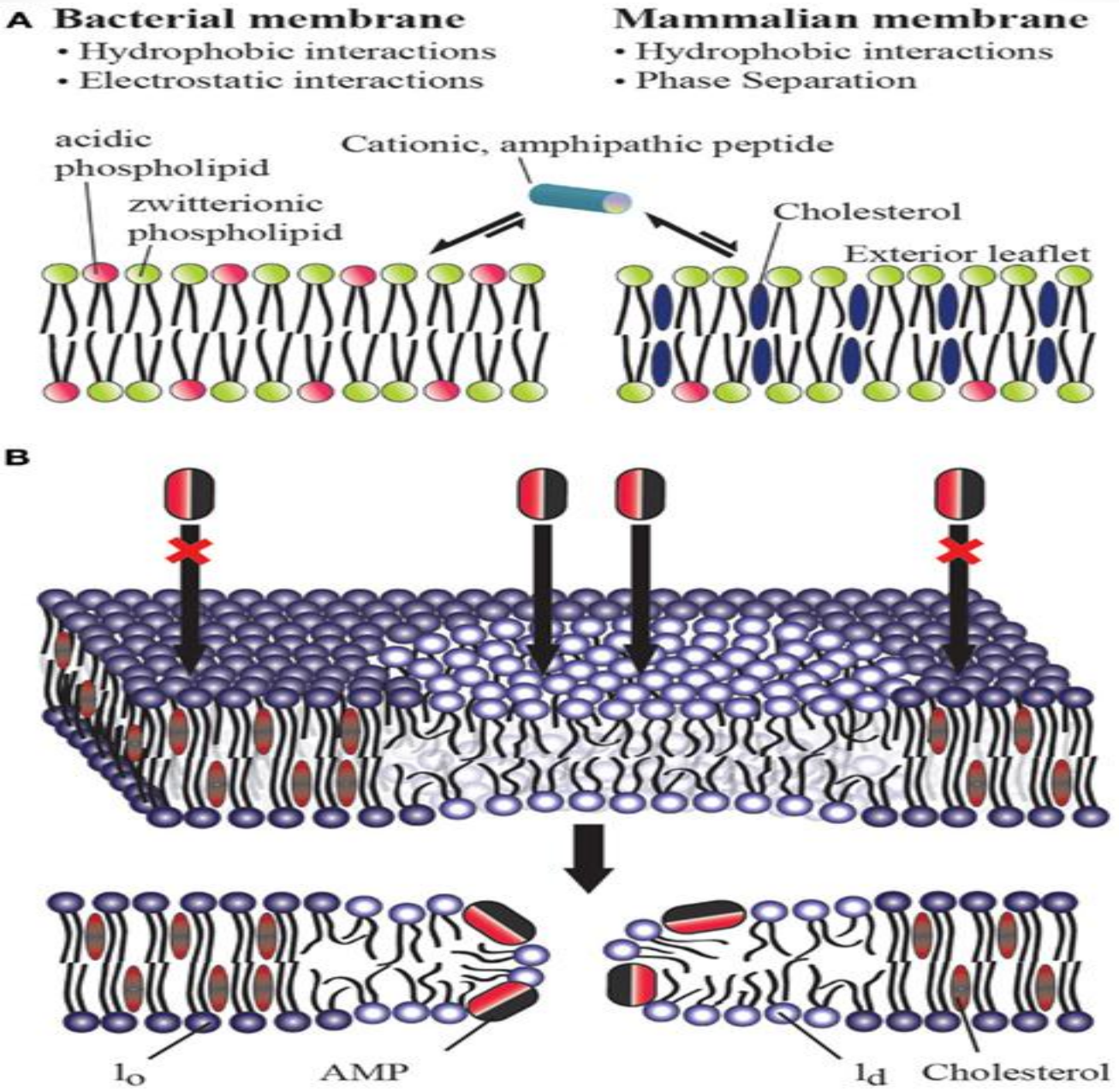

Antimicrobial peptide: What do they do?

- Permeabilize OM, IM

Channels

lysis

- D-analogs sometimes as effective

- Carry LPS
  - immune stimulation?

- Enzyme action
  - cell wall destruction
  - lipase , protease

-synergistic w/cationic peptides?

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Bacteriocin:bacterial AMPs

Bacterial AMPs are often referred to as bacteriocins.

Eukaryotic AMPs and bacteriocins share common features:

- Small in size (20-50aa)
- Cationic and amphiphilic or hydrophobic

Eukaryotic AMPs and bacteriocins differences:

- ❑ Bacteriocin are often very potent ,acting at pico-to nanomolar concentration,whereas micromolar concentration are reuired for the activity of eukaryotic AMPs.
- ❑ Most bacteriocin have a very narrow target spectrum,that is, being active against only a few species/genera closely related to the producer,whereas eukaryotic AMPs are generally less specific and target a large diversity of bacteria.
- ❖ Bacteriocin are considered as promising antimicrobial agents for different application,including :food preservation and infection treatment.
- ❖ Bacteriocin genes are located on mobile genetic elements ,such as:conjugative plasmids or transposons.

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Classification of bacteriocin

Bacteriocin from Gram –positive bacteria:

✓ Class I (Lantibiotics)

[19-38 aa containing post-translational modifications]

➢ Up to 11 subclasses have been suggested.nisin,subtilin,lacticin3147 and thuricinCD.

✓ Class II (Nonlantibiotics)

- [25-60aa ,cationic,heat-stable and nonmodifide peptides(exept for formation of disulfide bridges and circularization of cyclic peptides]

- bacteriocins of variable molecular weight,but usually small(<10kDa)

- Lactic acid bacteria(LAB) are frequently found as producer of class II bacteriocin.

✓ Class III

Big peptides,with molecular weight over 30 kDa.

In this class are helveticins J and V,acidofilicin A and lactacins A and B.

- Most of them have activity against species closely related to the producers but some has a wider inhibitory spectrum including pathogens such as listeria, staphylococci and some virulent species of bacilli, streptococci, clostridia and enterococci.

Bacteriocin from Gram –negative bacteria:

- Microcins(small peptides)

❖Class I microcins:small in size (<5kDa),contain extensive post –translational modifications. some members of this class are microcin C7,which is only seven amino acids, and microcin J25,which is 21 residues long.

❖Class II microcins: are larger(5-20kDa) and have little or no post-translational modification. This subclass includes microcin E492,colicin V,and H47.

- Colicins(larger proteins)

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Non-lantibiotics division

Divided into four subgroups

- Class IIa :
- peptides active against Listeria, (e.g. pediocinPA-1, sakacinP, enterocin A and P ,mesentericinY105 and leucocin A) containing lanthionine and β-lanthionine.

Class II b:

- Formed by a complex of two distinct peptides.(e.g. lactococcin G and plantaricins EF & JK)

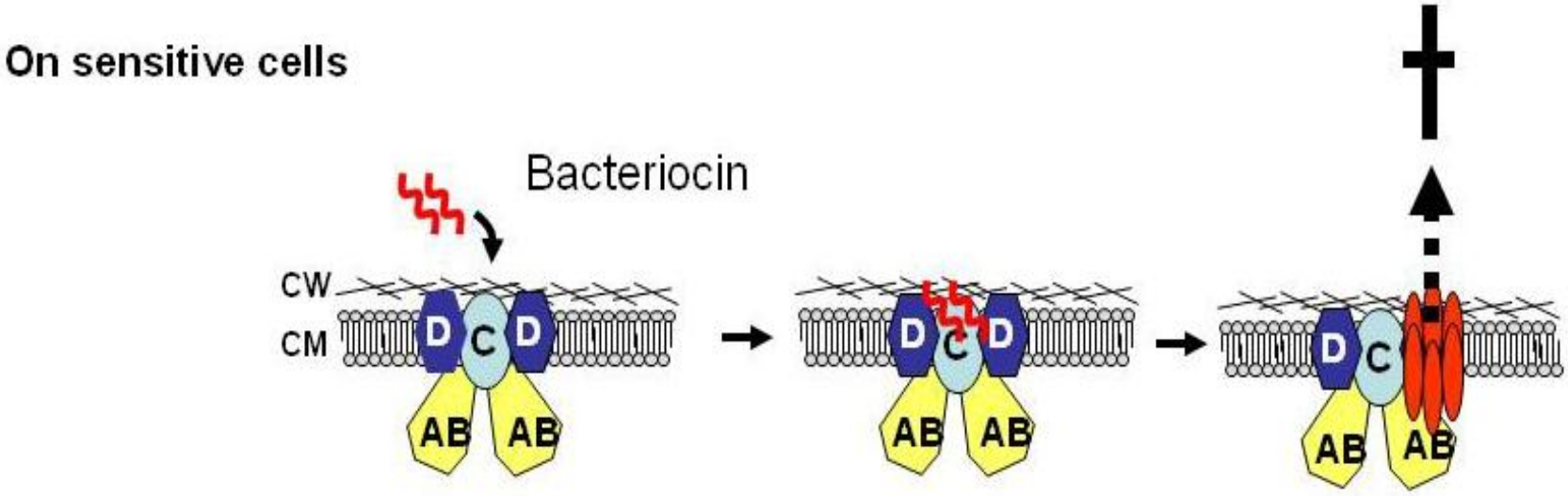
Class II c:

- Small peptides ,which are transported by leader-peptides. In this subclass are found only the bacteriocin divergicin A and acidocin B

Mechanisms of action of bacteriocins

i. Lipid II molecules in the membrane of target cells. Nisin and other lantibiotics.

ii. Membrane-located proteins(two transmembrane-located components (IIC and IID) and a cytoplamsic component (IIAB)) of the Man-PTS transporter. Class IIa bacteriocin.



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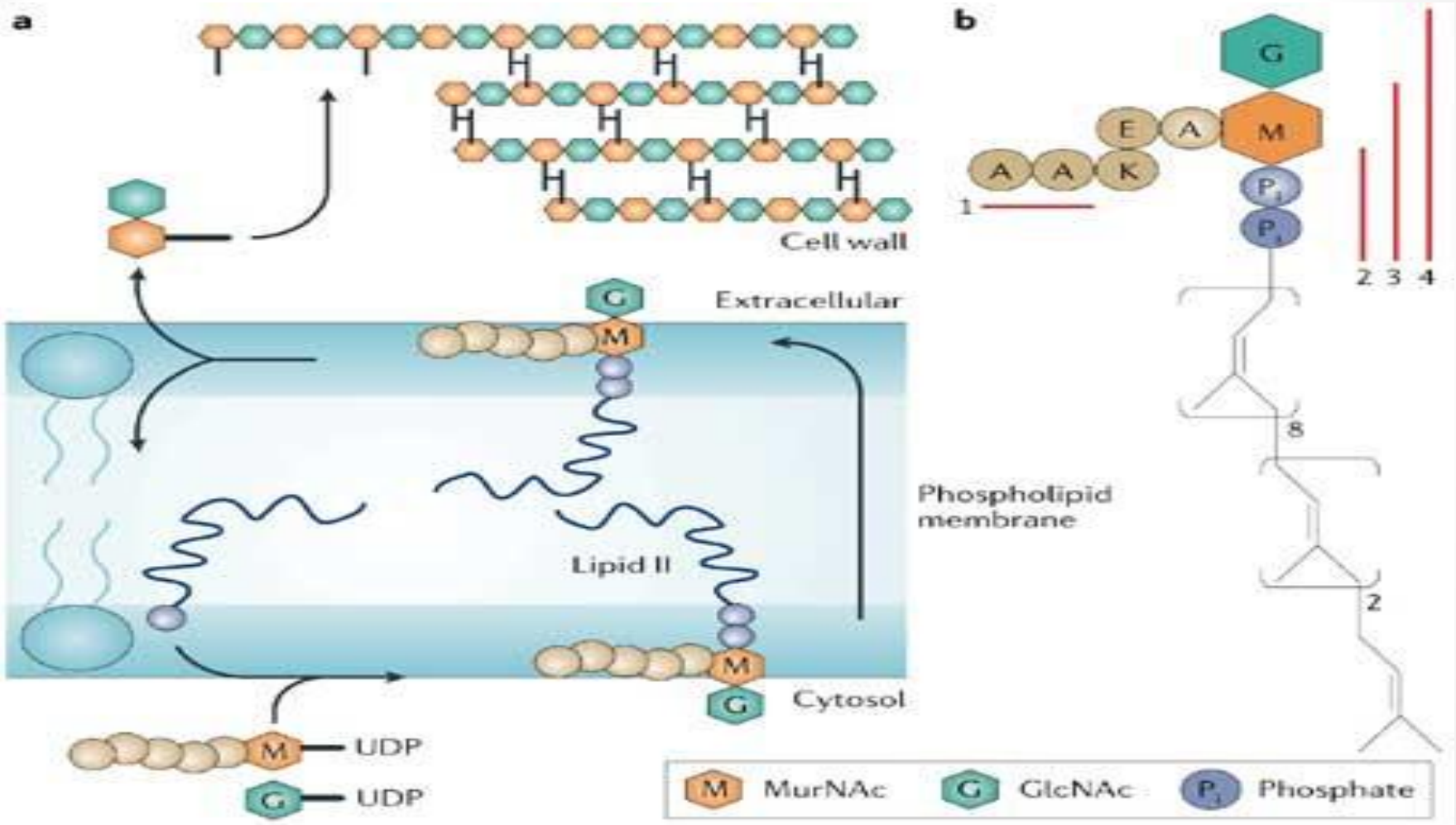

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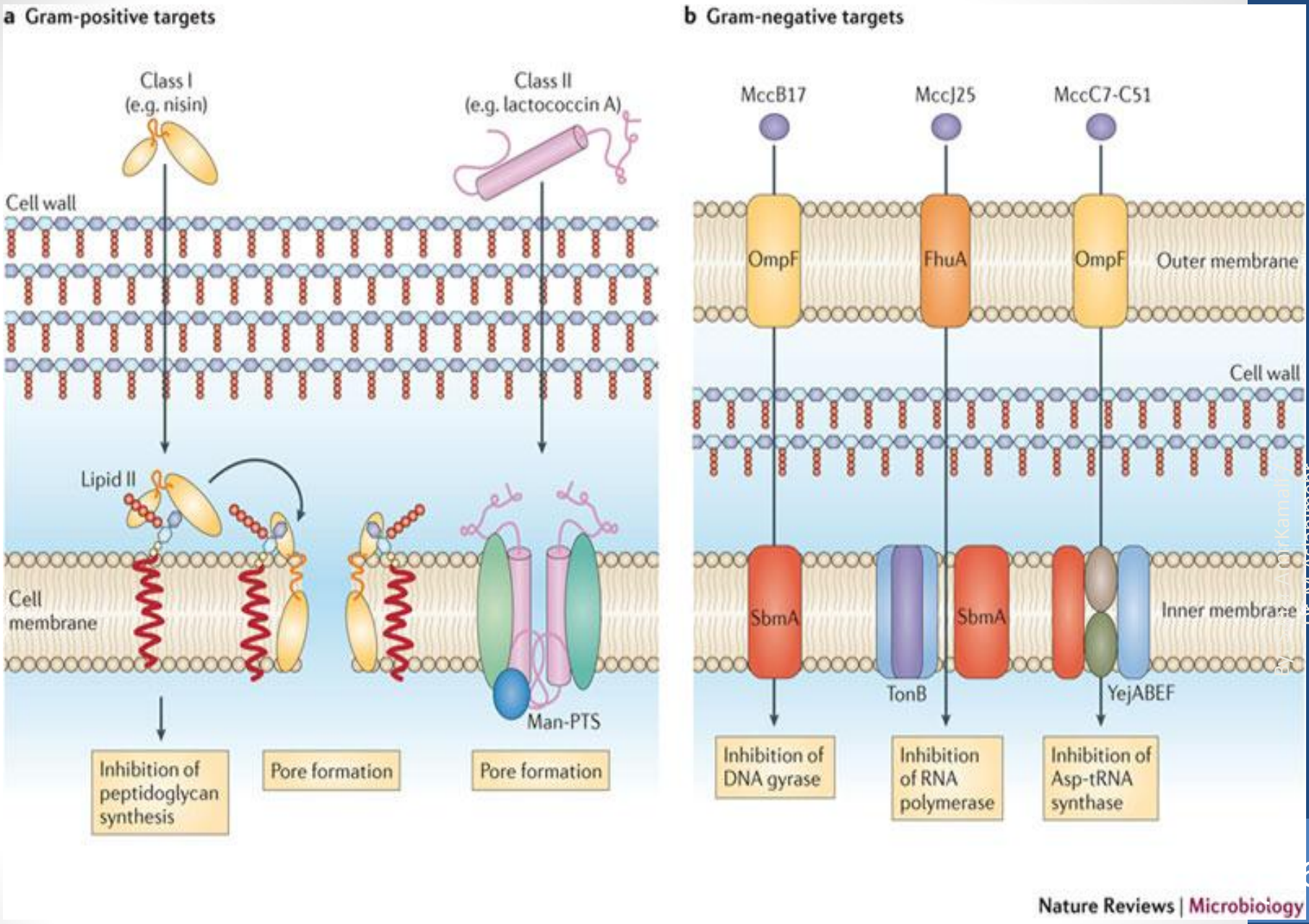
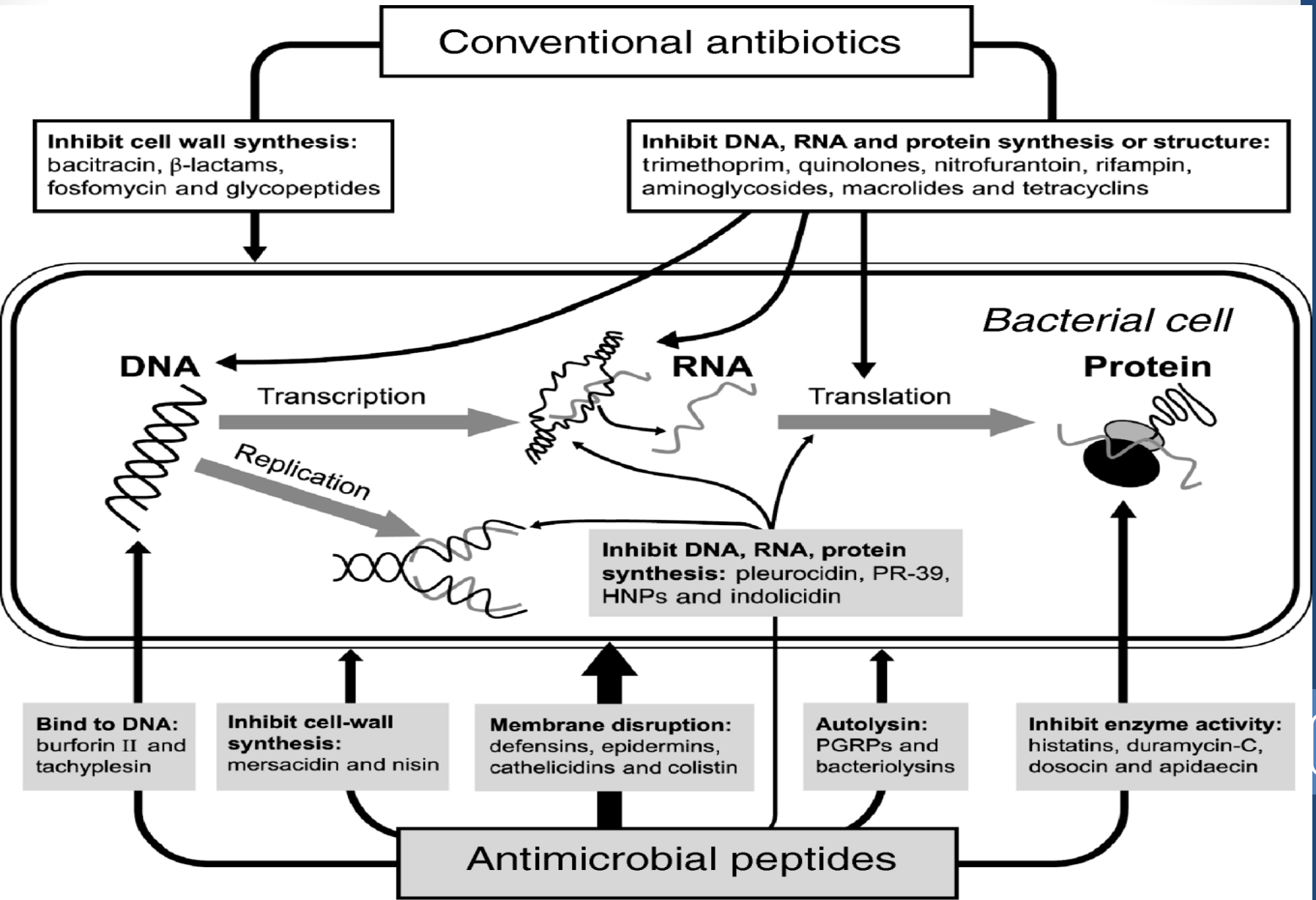


Mechanisms of action of conventional antibiotics and AMPs



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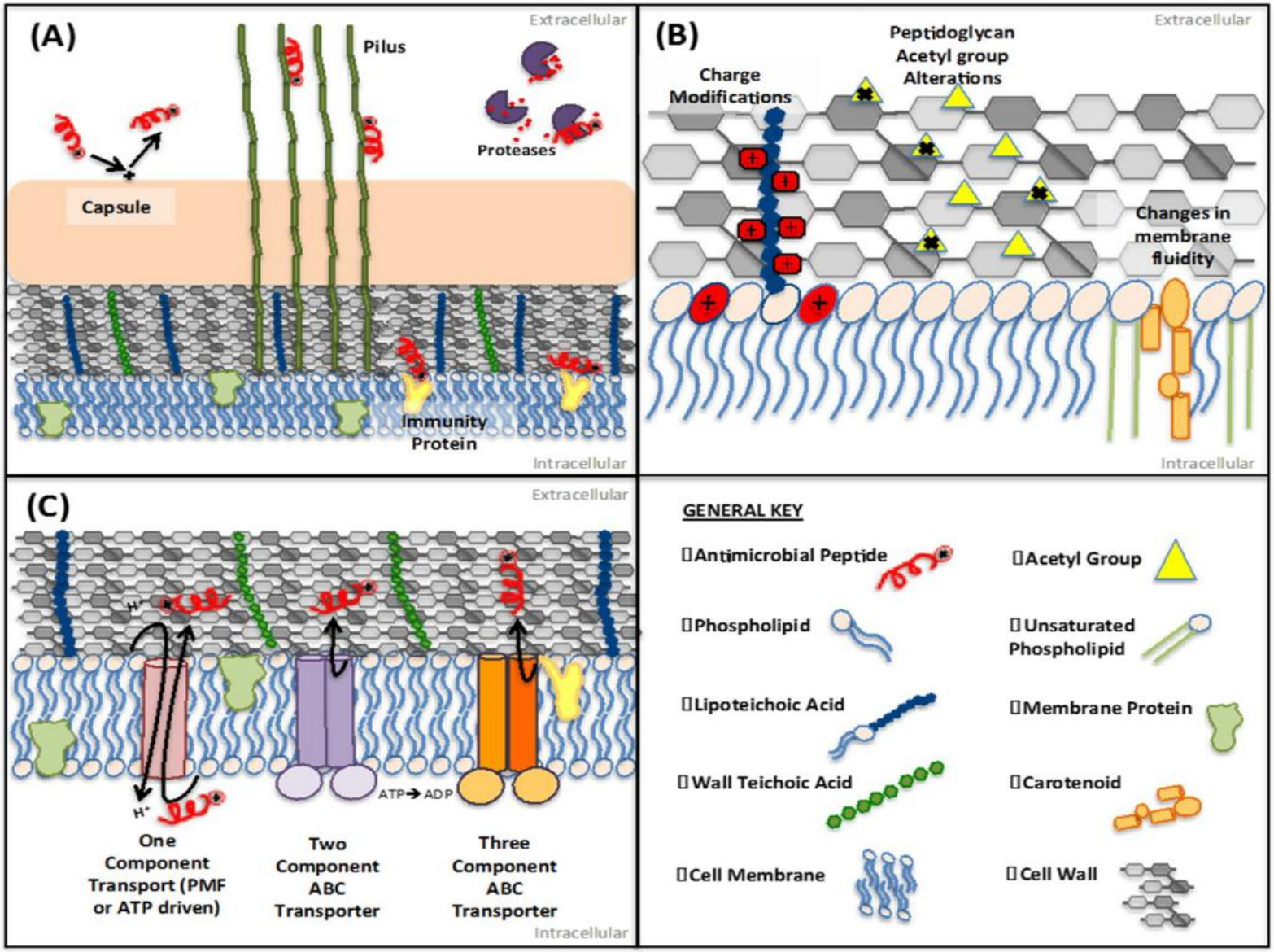
Antimicrobial peptide resistance machanisms in Gram positive bacteria



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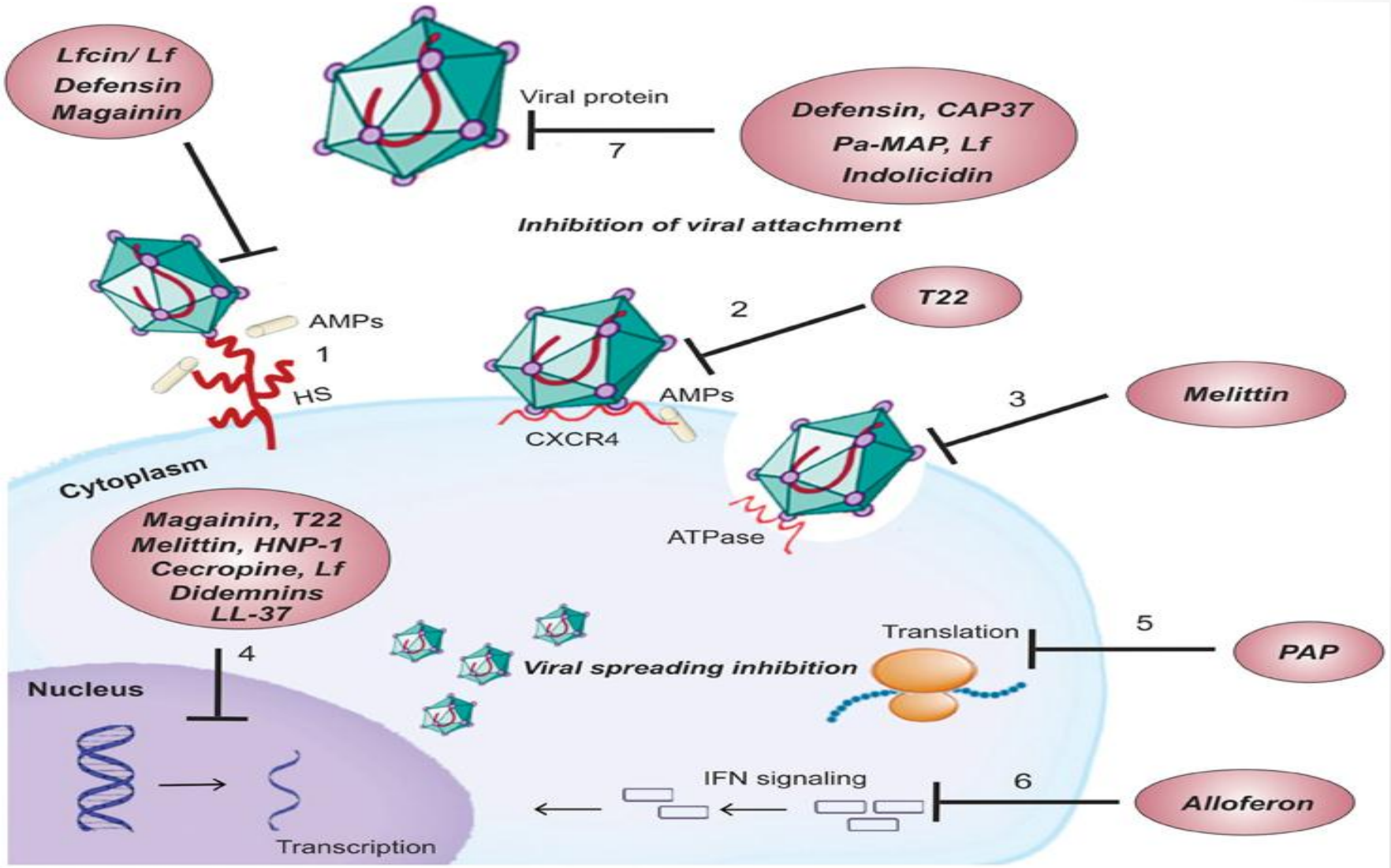
Mechanisms of action of cationic antiviral peptides



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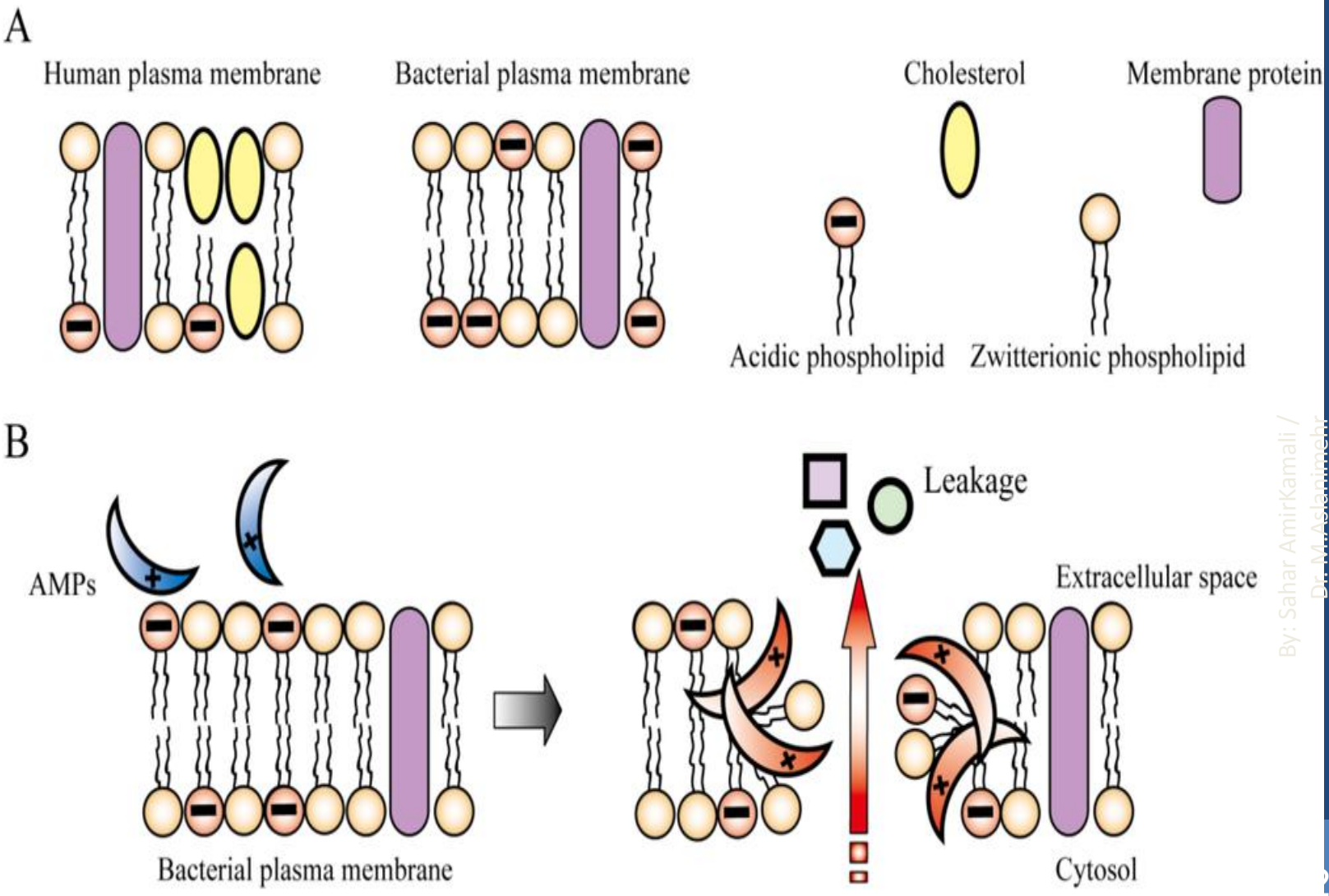
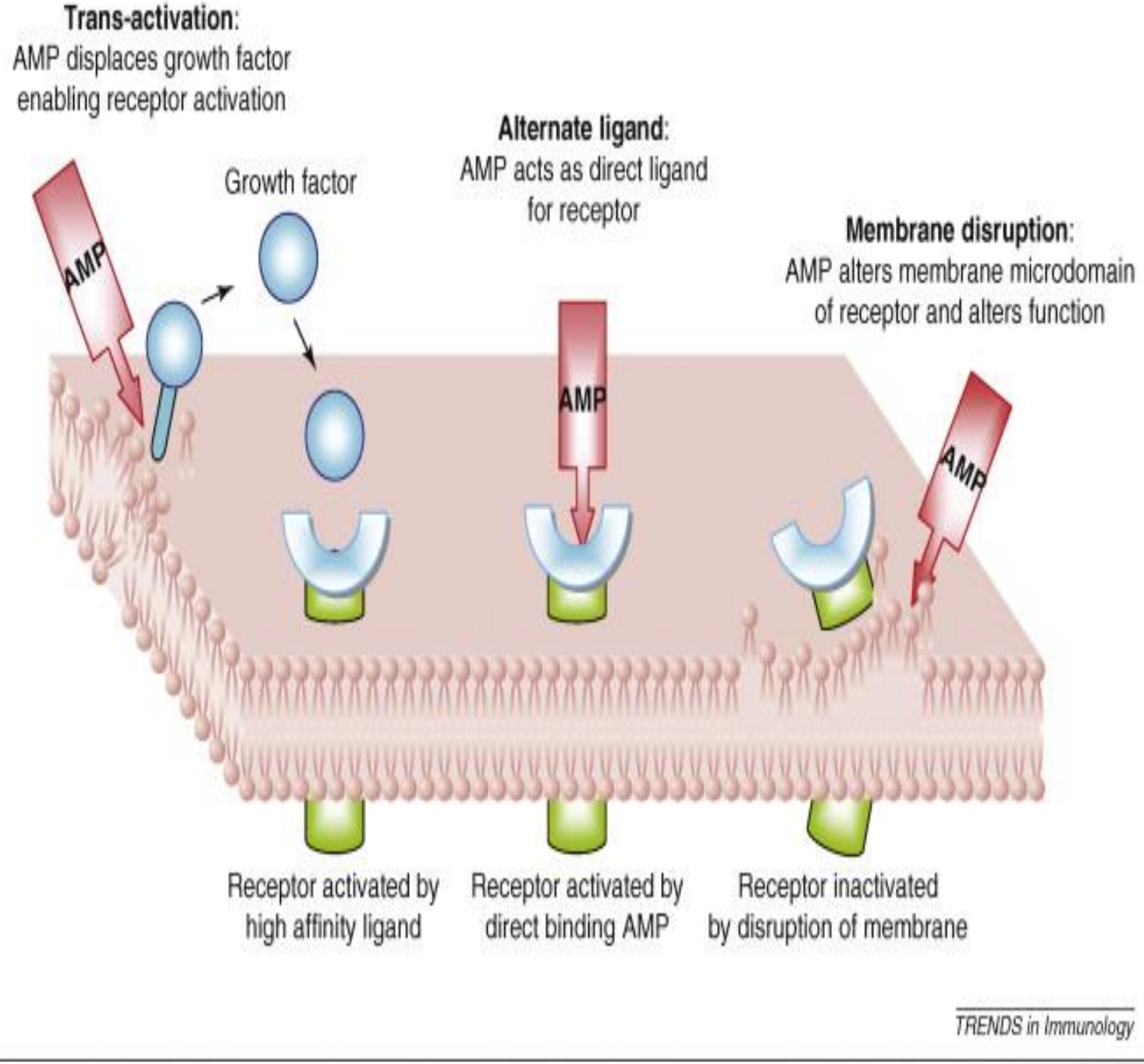
Cell surface targets: (1) Interaction of peptides with different glycosaminoglycan (e.g., HS) present on the cell surface competing with the virus for cellular binding sites. (2) Blocking of viral entry into the cell by binding the peptide to viral CXCR4co-receptor required for its entry. (3) Suppression of cell fusion by interfering with the activity of ATPase protein. Intracellular targets: (4) Suppression viral gene expression. (5) Inhibition of peptide chain elongation by inactivating the ribosome. (6) Activation of an immune modulatory pathway by induction of NK and IFN. Viral protein targets: (7) Binding of peptides to viral proteins causing inhibition of adsorption/virus-cell fusion.

Antimicrobial peptides, which are widely distributed in organisms across the evolutionary scale and are part of the innate immune systems in many of them, would be useful as prototype models for the design of new antimicrobial peptides

It is demonstrated that indolicidin induces filamentation in E. coli cells as a result of preferential inhibition of DNA synthesislike PR-rich peptides PR-39, bac-5 and bac-7 [10, 11]. All these peptides are known to inhibit macromolecular synthesis in bacteria. indolicidin does not lyse the cells. Indolicidin, from cytoplasmic granules of bovine neutrophilsIt consists of only 13 residues





Intracellular Targets

Inhibition of Cell Wall Synthesis

- Nisin
- Mersacidin
- defensin

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Inhibition of Nucleic Acid and Protein Synthesis

- Buforin II
- Temporins
- Cathelicidins
- Indolicidin
- Puroindoline
- bactenecins(Two members of this family, Bac5 and Bac7) present in bovine neutrophil granules.
- Microcin C (McC)(imported into bacterial cells by an outer-membrane porin and an inner-membrane ABC (ATP-binding-cassette) transporter. McC inhibits an essential aminoacyl-tRNA synthetase
- Microcidin B17 is a modified bacterial peptide that targets DNA gyrase penetration into bacterial cell is facilitated by the inner-membrane protein SbmA.

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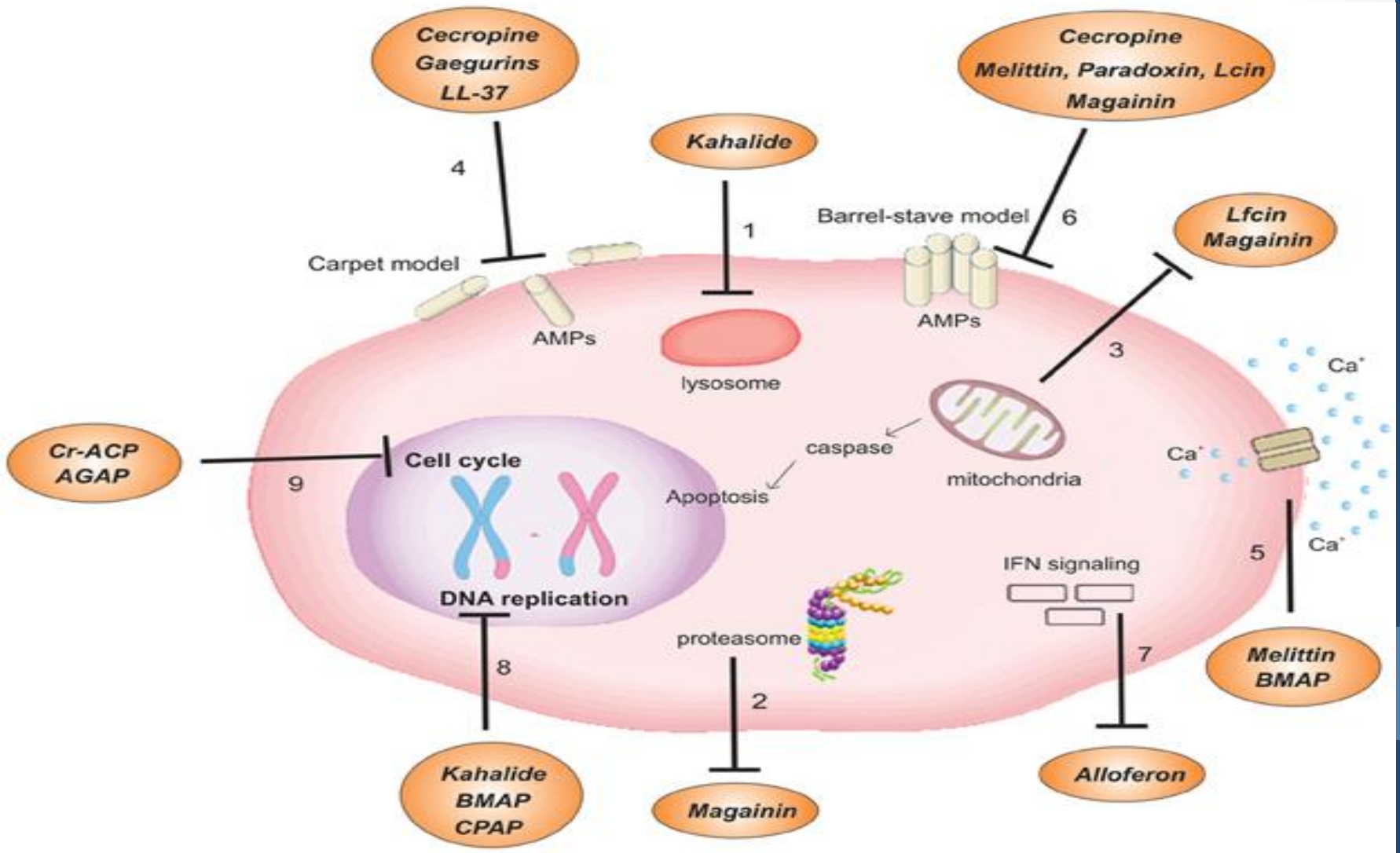
Mechanisms of action of cationic antitumor peptides



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Cationic antiviral peptides have been isolated from various sources and present broad antiviral activities against several viruses with different antiviral mechanisms of action . They can either inhibit viral attachment by binding to viral targets on the host cell surface, or target viral proteins, therefore blocking viral fusion and entry into the host cell. Another mechanism of action is intracellularly driven where spreading of the virus is inhibited through the suppression of viral gene expression, inhibition of translation or by immune modulatory activities

By: Sahar AmirKamali / Dr. M.Aslanimehr

Cationic Antiviral Peptides Isolated from Vertebrates

- In this group are included defensins, Lfcin and LL-37

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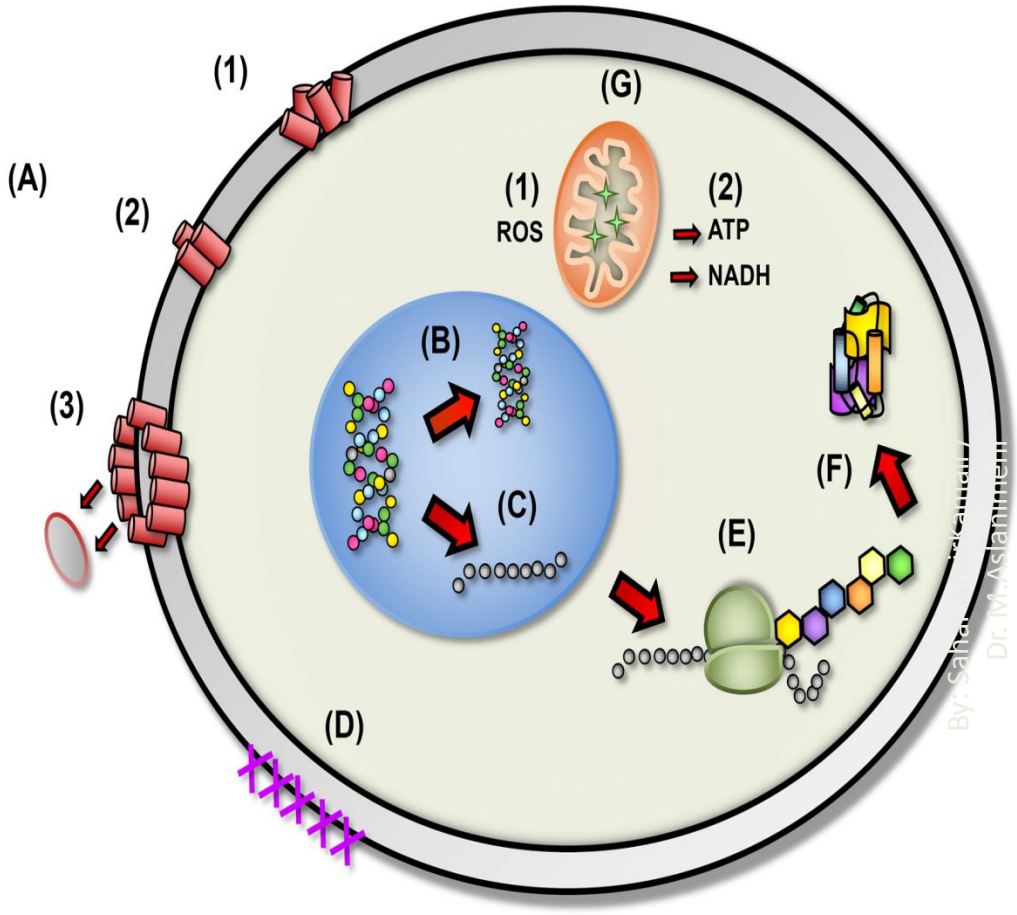
Cationic Antiviral Peptides Isolated from Invertebrates

- melittin, cecropin, and alloferon
- melittin has been reported to have inhibitory activity against enveloped viruses such as HIV-1, HSV-2, and the Junin virus (JV), an arenavirus.
- A proposed mechanism of action has suggested that melittin suppresses cell fusion mediated by HSV-1 syncytial mutants probably by interfering with the activity of the Na+ K+ ATPase, a cellular enzyme involved in the membrane fusion process

(A) Disruption of cell membrane integrity: (1) random insertion into the membrane, (2) alignment of hydrophobic sequences, and (3) removal of membrane sections and formation of pores. (B) Inhibition of DNA synthesis. (C) Blocking of RNA synthesis. (D) Inhibition of enzymes necessary for linking of cell wall structural proteins. (E) Inhibition of ribosomal function and protein synthesis. (F) Blocking of chaperone proteins necessary for proper folding of proteins. (G) Targeting of mitochondria: (1) inhibition of cellular respiration and induction of ROS formation and (2) disruption of mitochondrial cell membrane integrity and efflux of ATP and NADH



Schematic representation of AMP resistance mechanisms. (A) Gram-positive bacteria resist AMPs via teichoic acid modification of LPS molecules and L-lysine modification of phospholipids. (B) Gram-negative bacteria resist AMPs by modifying LPS molecules with aminoarabinose or acylation of Lipid A unit of LPS molecules. (C) Bacteria express some positively charged proteins and integrate them in the membrane so positive charges repulse each other and bacteria can resist such AMPs. (D) Bacteria produce negatively charged proteins and secrete them into extracellular environment to bind and block AMPs. (E) The

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