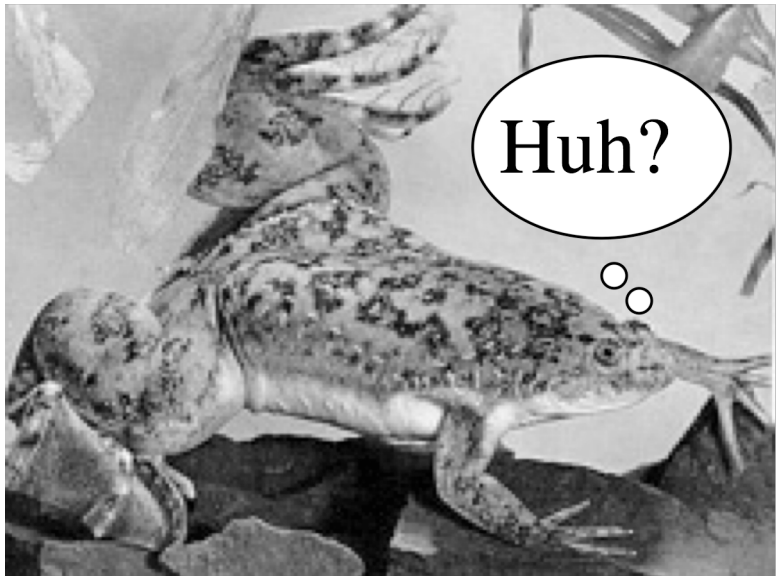
Antimicrobial Peptides

An ancient immune system?

or

This frog could save *your* leg



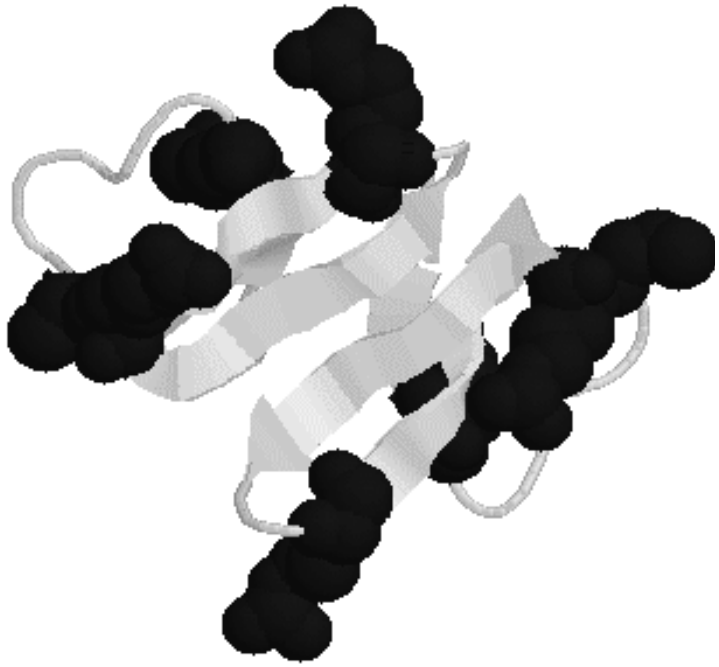
Antimicrobial peptides: Where are they?

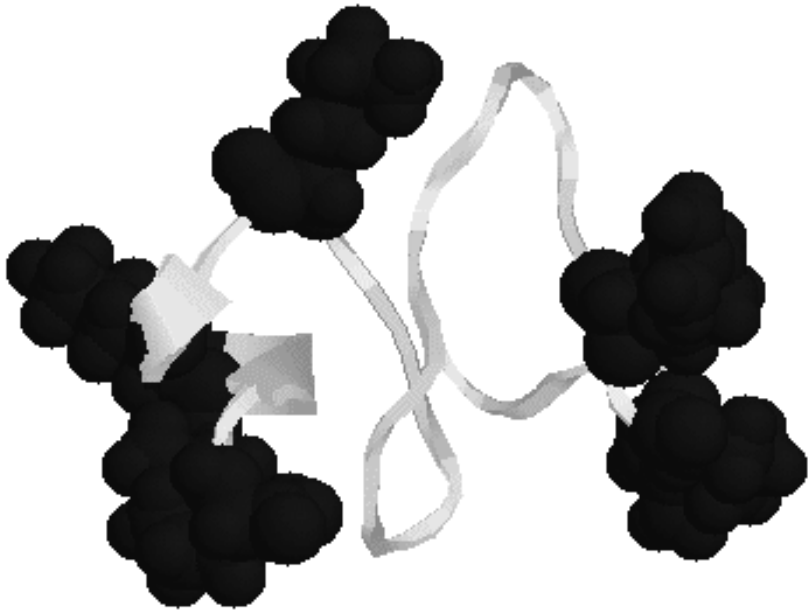
- *In everything from Amoebas to Humans*
- *Abundant in vertebrates in:*
  - *External Mucosa*
    - *eyes, mouth, genitourinary, skin, lung, trachea*
  - *Immune cells*
    - *neutrophils*
  - *Intestinal tract (duodenum)*
    - *in humans; Paneth cells are the source*

Antimicrobial peptides: What are they?

- *Cationic, Amphipathic*
  - *Linear α-helical (20-35 AA)*
    - *Magainin, cecropins, histatin*
  - *Pro-Arg rich (more polar)*
    - *bac 5 and 7 (30-50 AA; 70% pro/arg)*
  - *Disulfide Rich α-structure*
    - *amoebapores, NK-lysin*
  - *Disulfide Rich β-structure*
    - *Defensins: three or more disulfide (30-70 AA)*
    - *"Loops" (11-22 AA)*
      - *tachpyylesins, protegrins ,ranalexin, polymyxin*

Defensin-like

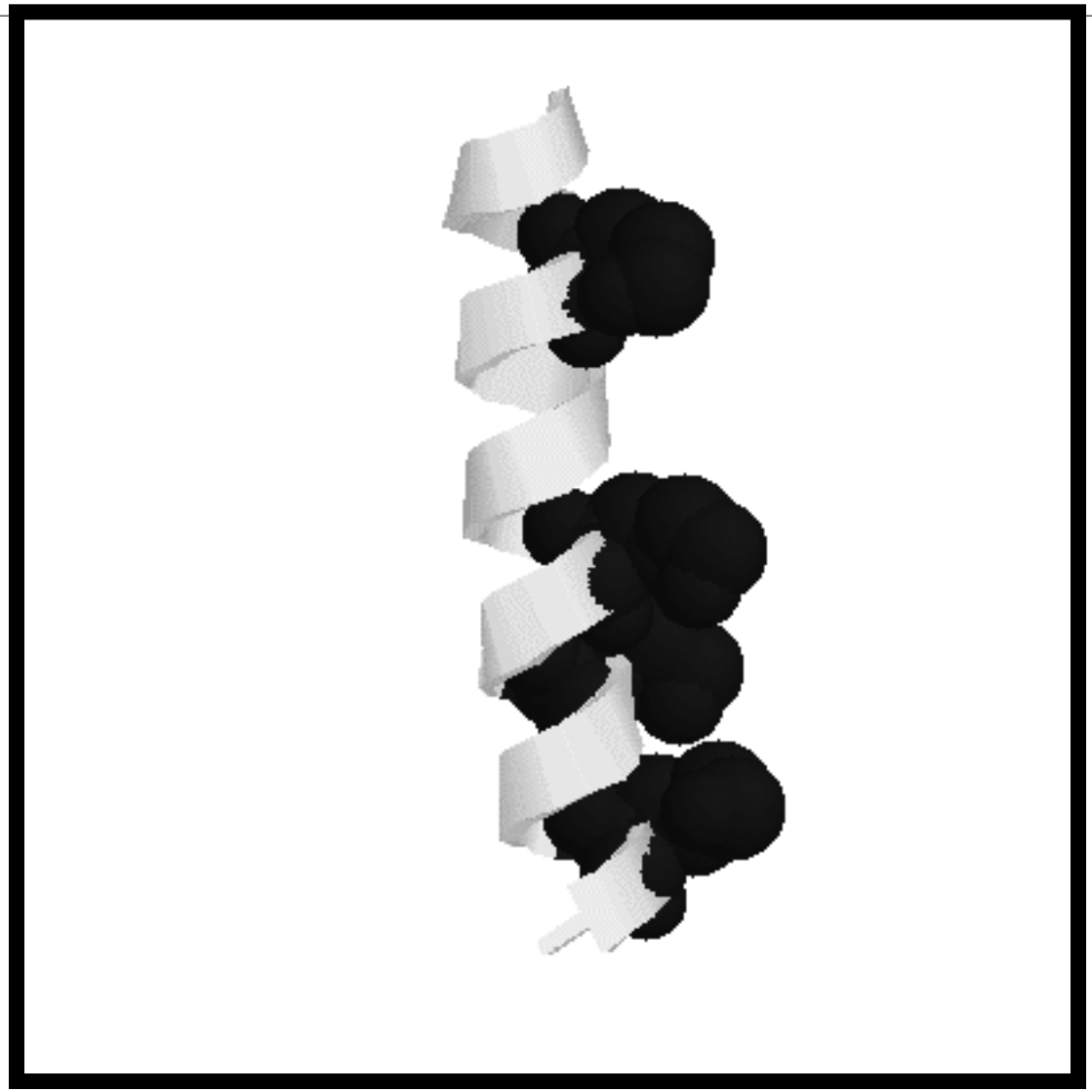




HUMAN

PLANT

Helical: PGLa(frog)



Antimicrobial peptides:

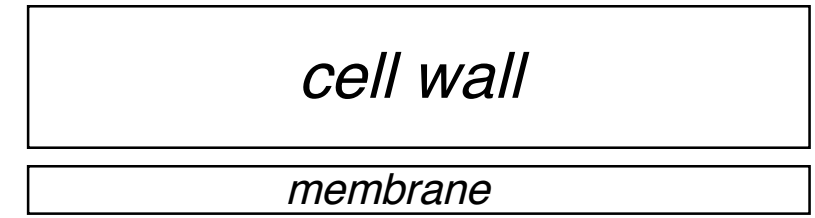
What are they?

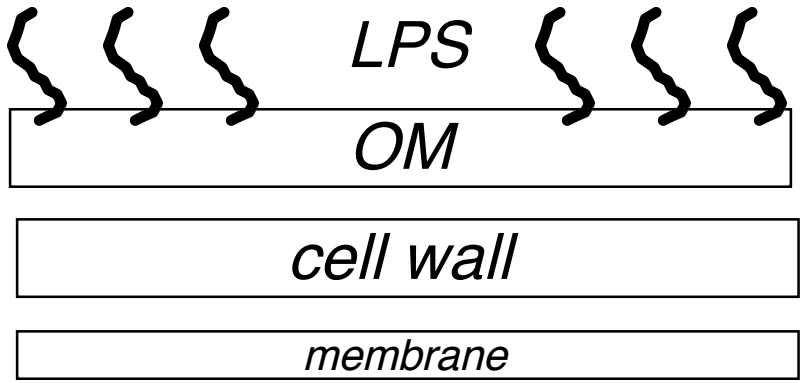
- *Small Proteins*
  - *lysozyme*
  - *phospholipase a*
  - *cathepsins*

Antimicrobial peptides:

What do they do?

- *Antifungal*
- *Antibacterial*
  - *Gram positive*
  - *Gram negative*





Antimicrobial peptides: 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?*

Mechanisms?

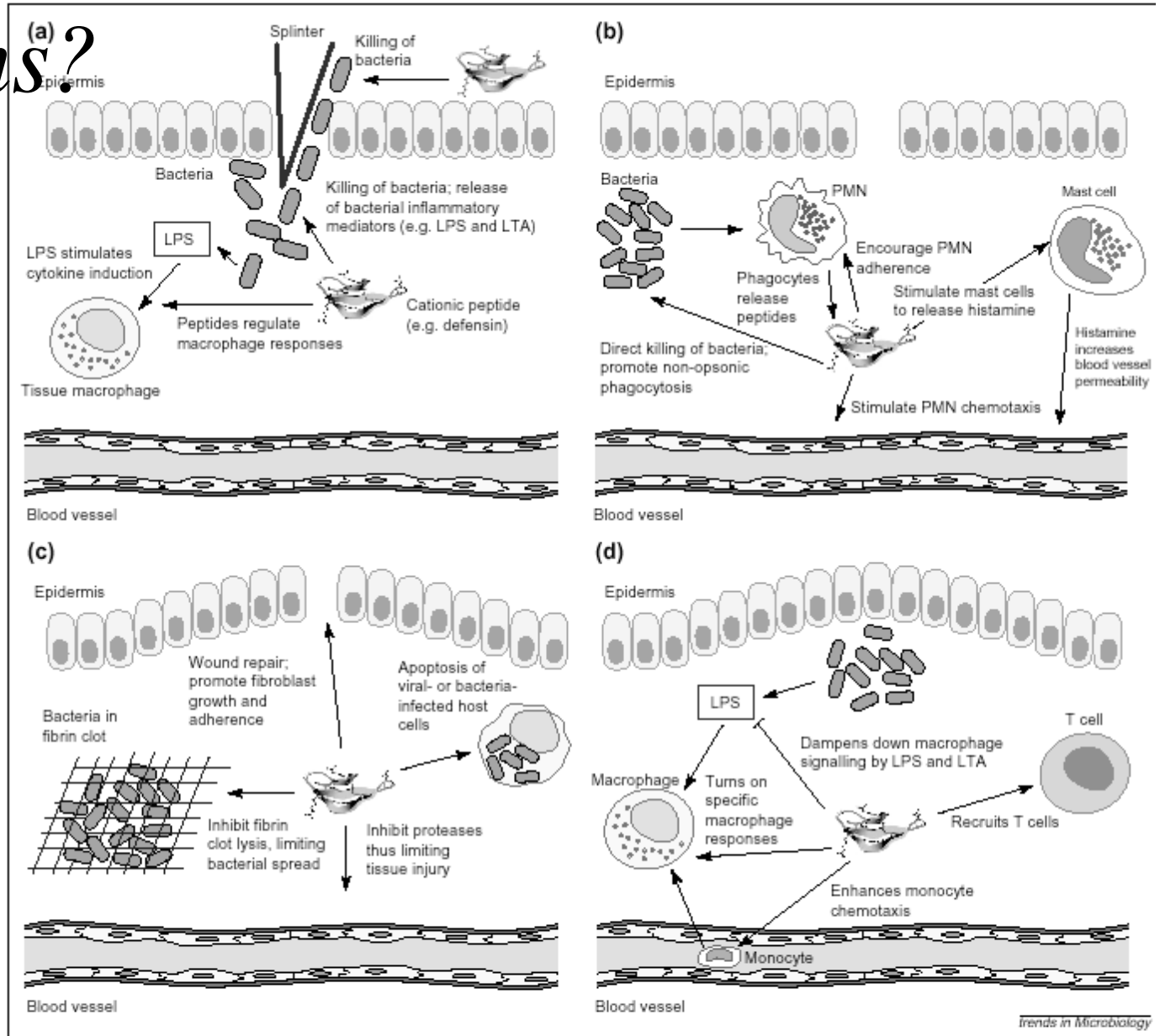
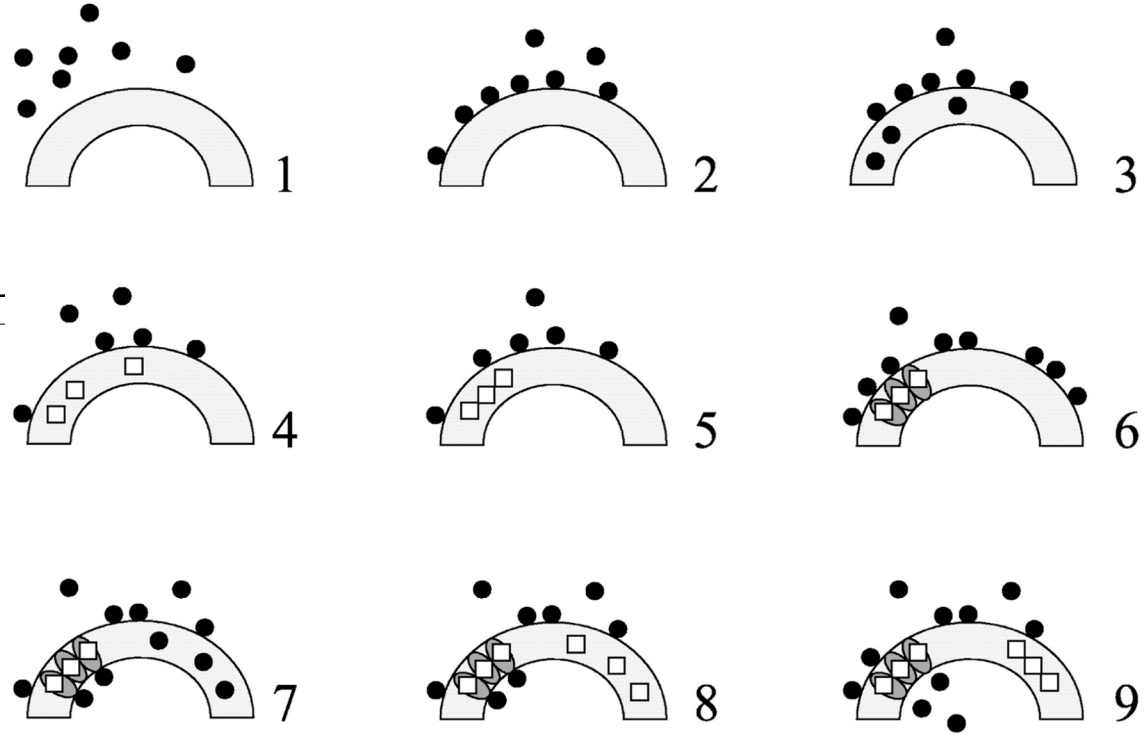


Fig. 3. Proposed role of antimicrobial peptides in acute **(a–c)** and chronic **(d)** inflammation. The specific roles of individual peptides (shown as the molecular structure of human defensin 1) in the various events depicted have been demonstrated for individual separate natural peptides (Table 3) but have not been demonstrated with any single peptide. Thus the overall scheme presented is a chimera of these separate effects. Abbreviations: LPS, lipopolysaccharide; LTA, lipoteichoic acid; PMN, polymorphonuclear leukocyte.

Mechanisms: Latest

http://pharmrev.aspetjournals.org/cgi/content/full55/1/27

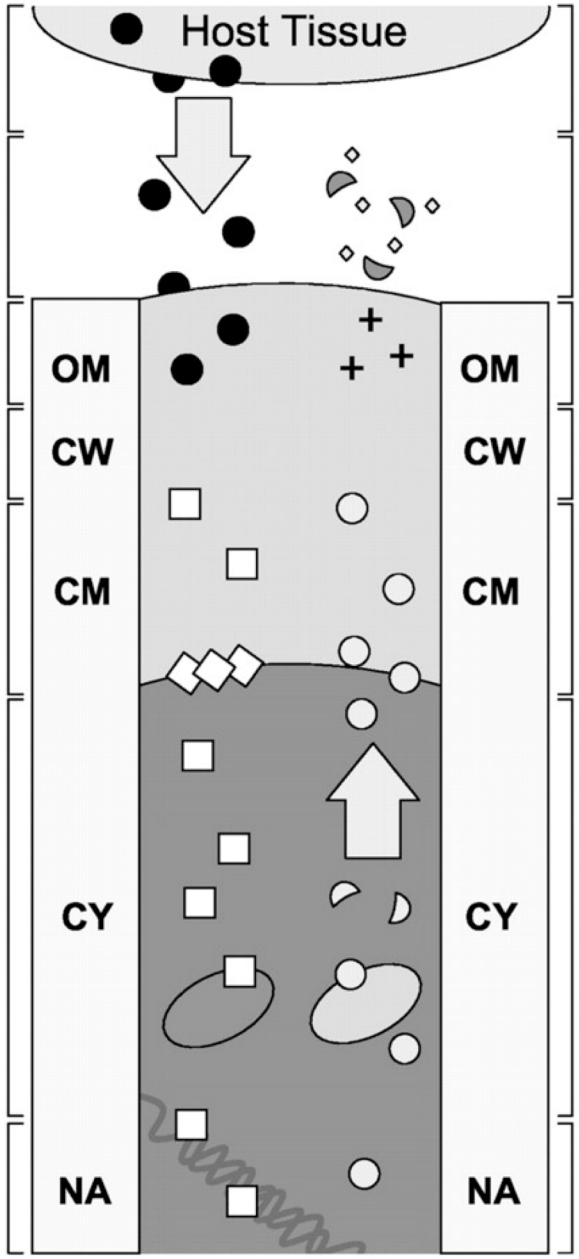


Integrative model of antimicrobial peptide mechanisms of action. Recurring themes in antimicrobial peptide mechanisms and the sequence of events associated with inhibition or killing of pathogens include: 1) initial electrostatic and hydrogen bond attraction; 2) subsequent hydrophobic interactions as the peptides contact the target surface; 3) accumulation and threshold concentration driving initial peptide conformational dynamics and early membrane deformation; 4) further peptide conformational phase transition and insertion within the membrane core; 5) self-association and multimerization; 6) formation of quaternary peptide complexes such as barrel-stave or toroid pore configurations; 7) translocation of peptide to the inner facet of the cytoplasmic membrane; 8) ongoing peptide accumulation and interactions as described above; and 9) access and targeting of essential intracellular structures and functions. Native antimicrobial peptides are indicated as dark spheres, whereas activated or conformation-transformed peptides are depicted as light squares.

Mechanisms:Latest

Mechanism of Action

- **Context-Specific Immunity / Synergy** (peptides function optimally in specific contexts)
- **Perturb Capsule / Immunovulnerable** (opsonization of encapsulated pathogens)
- **Protease-Resistant Structure(s)** (amide – indolicidin; compact form - defensins)
- **Target Electronegative Components** (polymyxin B – Gram-negative Lipid A)
- **Interfere with Cell Wall Integrity** (lantibiotics – Gram positive peptidoglycan)
- **Target Electronegative Phospholipids** (cationic peptides – bacterial pathogens)
- **Exploit Prokaryotic $\Delta\psi$** (self-promoted uptake – bacteria)
- **Conformer Transition / Multimerization** (many antimicrobial peptides)
- **Intracellular Access / Import** (likely many antimicrobial peptides)
- **Target Specific Intracellular Ligand** (DNA gyrase – microcin B17)
- **Protease-Resistant Structure(s)** (many antimicrobial peptides)
- **Target Intracellular Organelle(s)** (histatins – mitochondrial affinity in fungi)
- **Target Specific Nucleic Acid Motifs** (hypothetical)
- **Synergistic / Combinatorial Effects** (hPF-4 + CTAP-3; magainin + PGLa)



Mechanism of Resistance

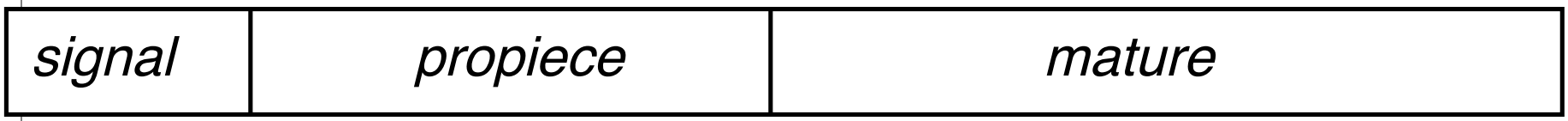
- **Niche-Specific Immunoavoidance** (renal / osmotolerance – *Staphylococcus*)
- **Anionic Capsule Shielding** (alginic acid – *Pseudomonas*)
- **Extracellular Degradation (Proteases)** (PgtE – *Salmonella; pla* gene product – *Yersinia*)
- **Amidate Lipid A Constituents** (aminoarabinose – *Salmonella*)
- **Modify Cell Wall Pathways or Precursors** (hypothetical)
- **Modify Cell Membrane Phospholipids** (lysyl-phosphatidylglycerol – *Staphylococcus*)
- **Assume SCV Phenotype** (reduce $\Delta\psi$ - *Staphylococcus*)
- **Preclude Phase Transition / Multimerization** (hypothetical)
- **Peptide Efflux / Antiport** (MtrCDE – *Neisseria;* RosA/RosB – *Yersinia*)
- **Modify Specific Intracellular Target** (ΔDNA gyrase B subunit – *Escherichia*)
- **Intracellular Degradation (Proteases)** (hypothetical)
- **Organelle Modification** (mitochondrial adaptation in fungi)
- **Mutate Base Pair Sequences** (hypothetical)
- **Coordinate Adaptation** (*PhoQ/PhoP* regulon – *Salmonella)*

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!*

Antimicrobial peptides:
How are they made?

- *NFκB-type genetic element seems universal*
  - *like acute phase response in humans*
- *Protective anionic piece is common,*



Antimicrobial peptides: New Clinical Antibiotics?

- *Indolicidin analog fights test infections*
  - *IP or IV*
- *Protegrin analog*
  - *helps chemotherapy oral mucositis*
- *Magainin analog, LOCILEX™ Cream underwent clinical trials (now Genaera Corporation)*
  - *diabetic ulcers, not approved*
  - *Squalamine, amino sterol cationic peptide analog-anti-obesity and anti angiogenesis*

Antimicrobial peptides: Problems with use?

- *Proteolysis, Salt, Transport properties*
- *Manufacture*
  - *chemical synthesis is very expensive;$100's/gram!*
  - *organic mimics or modular design may help*
  - *May be possible to produce in bacteria or plants*
    - *protective piece for bacteria*
    - *TMV with tobacco (GENEWARE®)*

Antimicrobial peptides: References

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5. *Tang YQ, Yeaman MR and Selsted ME (2002) Antimicrobial peptides from human platelets. Infect Immun 70: 6524-6533*