Virulence factors of *Staphylococcus aureus*: molecular aspects

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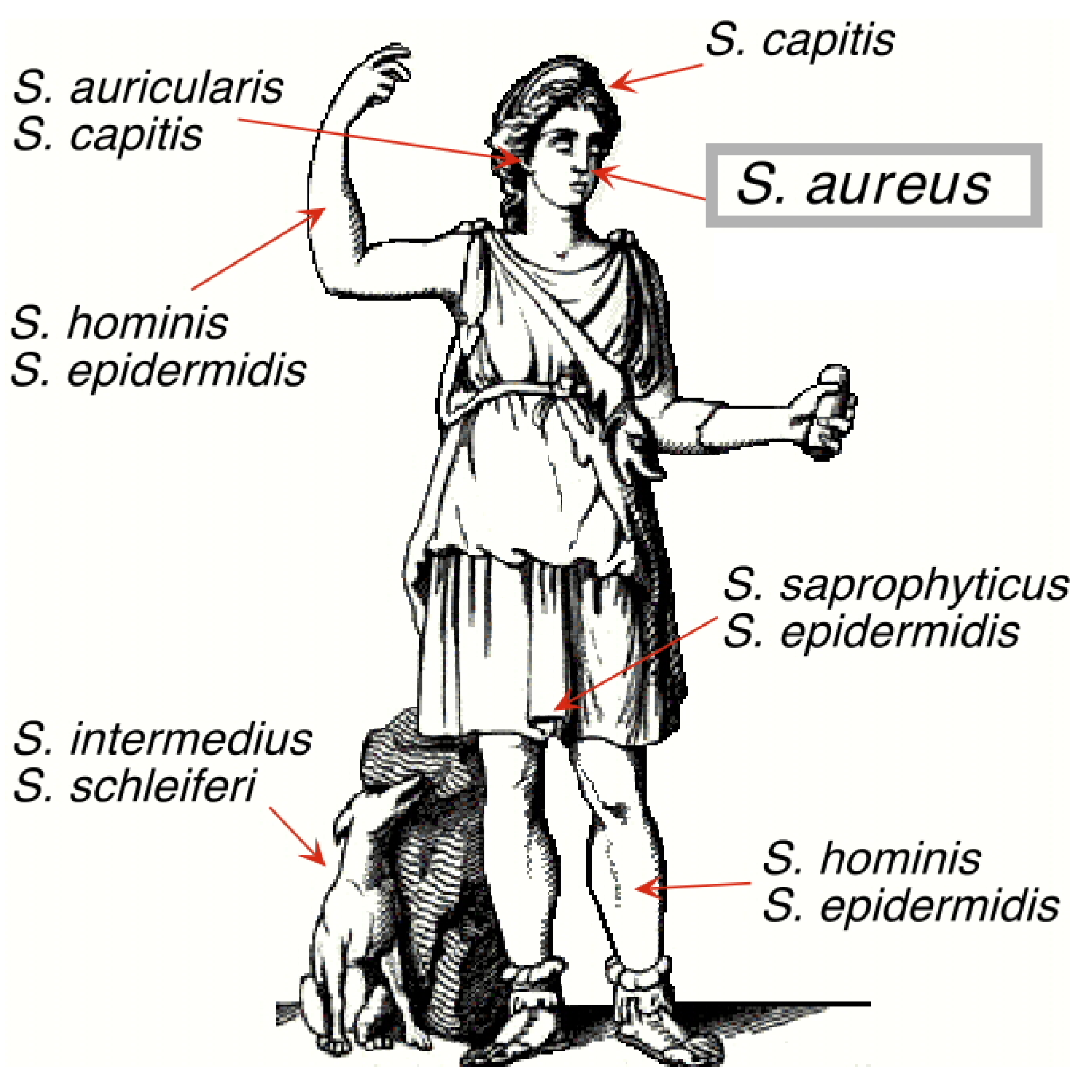
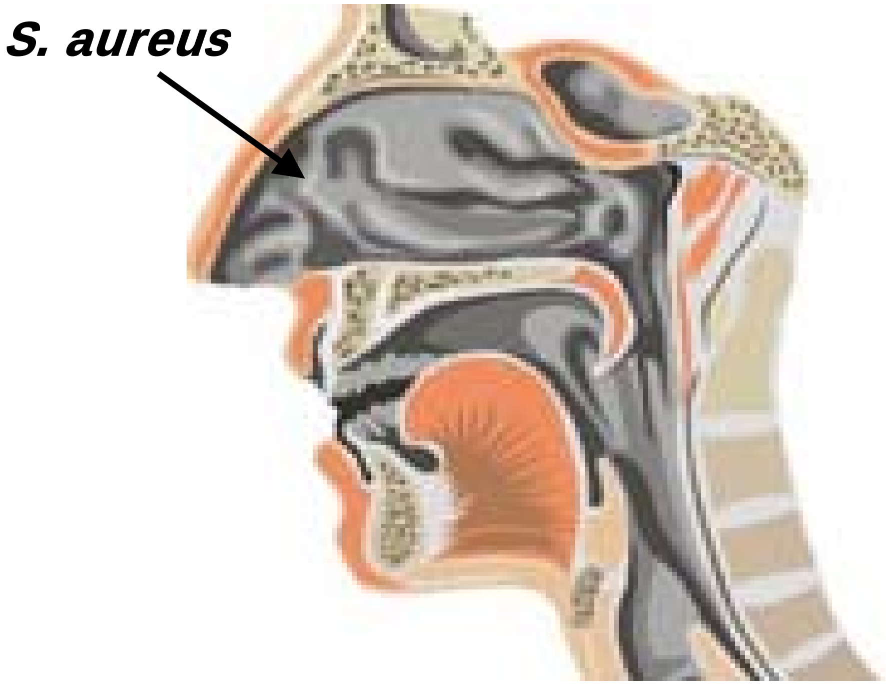
Mammalian skin - the staphylococcal ecosystem

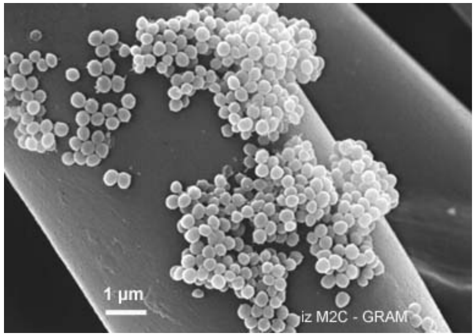


Risk factors for *S. aureus* infections:

- **Nasal colonization** (30-40% of the population)
- **Use of catheters** and artificial implants (Biofilm formation)
- **Immunosuppression** (Chemotherapy, CGD, cystic fibrosis, AIDS)

S. aureus infections

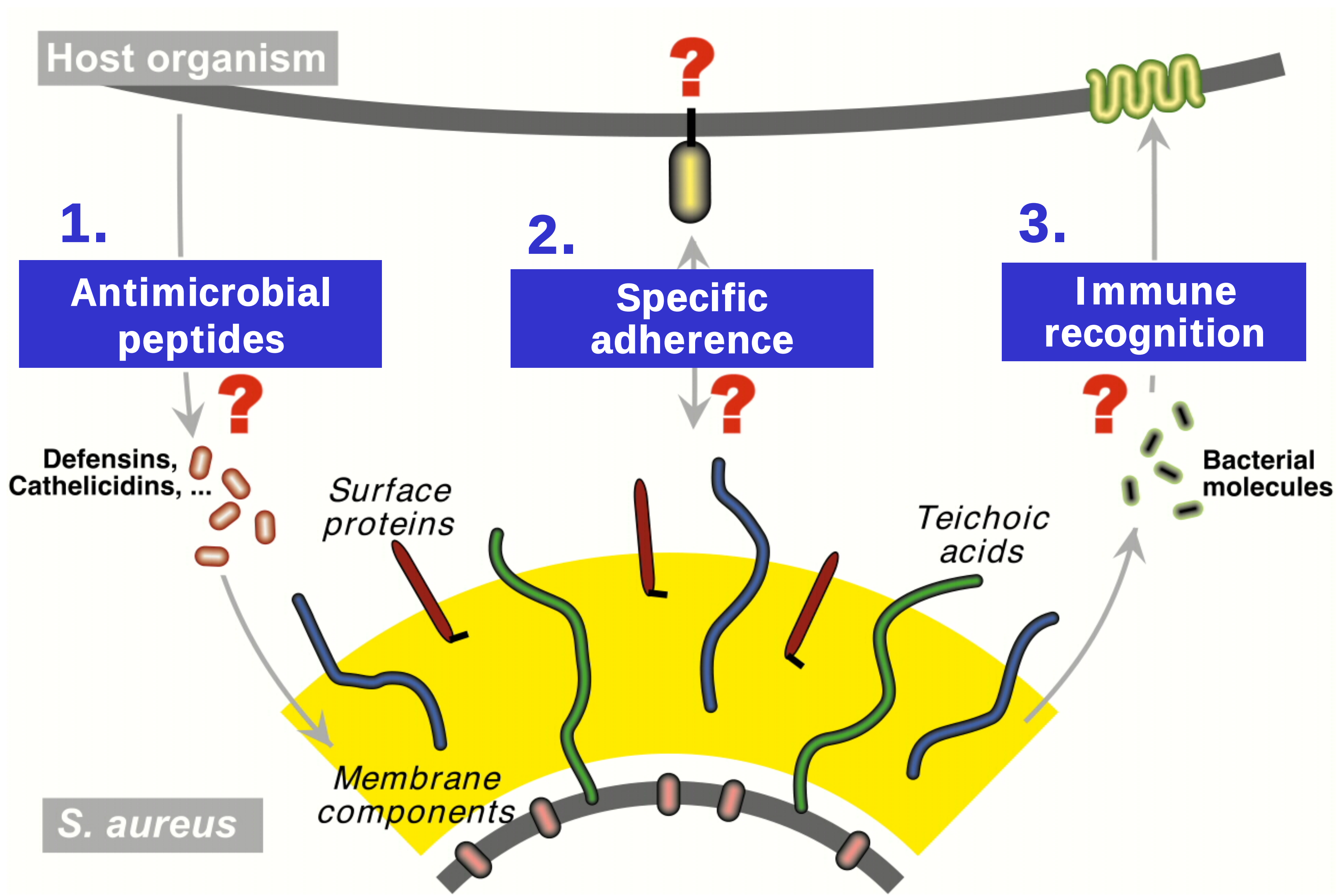




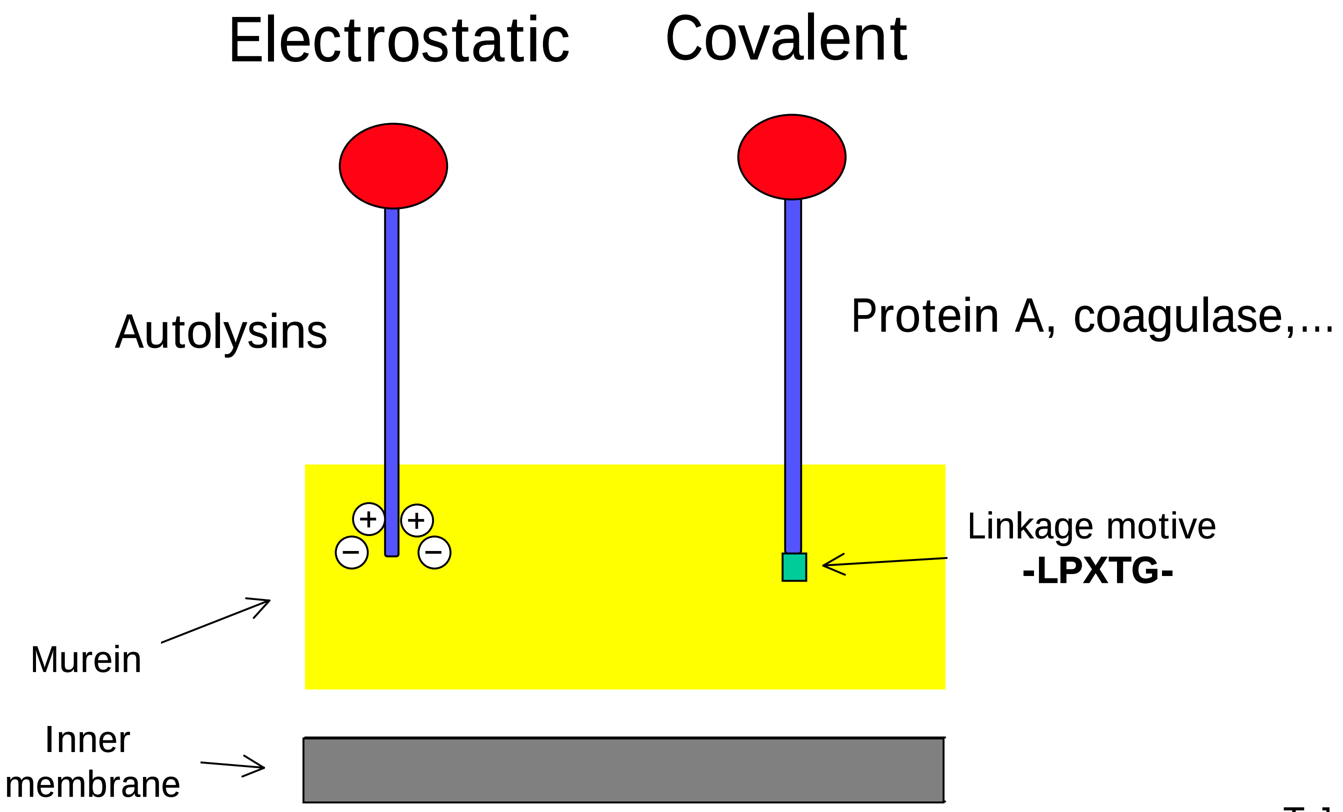
Infected implant

- Skin and **wound infections**
- Catheter and **device-related infections**
- 40% of **nosocomial infections**
- **Sepsis**, septic shock
- More than **30.000 deaths** per year (USA)
- Multiple antibiotic resistance (**MRSA, VRSA**,...)

Host / pathogen interaction



Covalent attachment of surface proteins

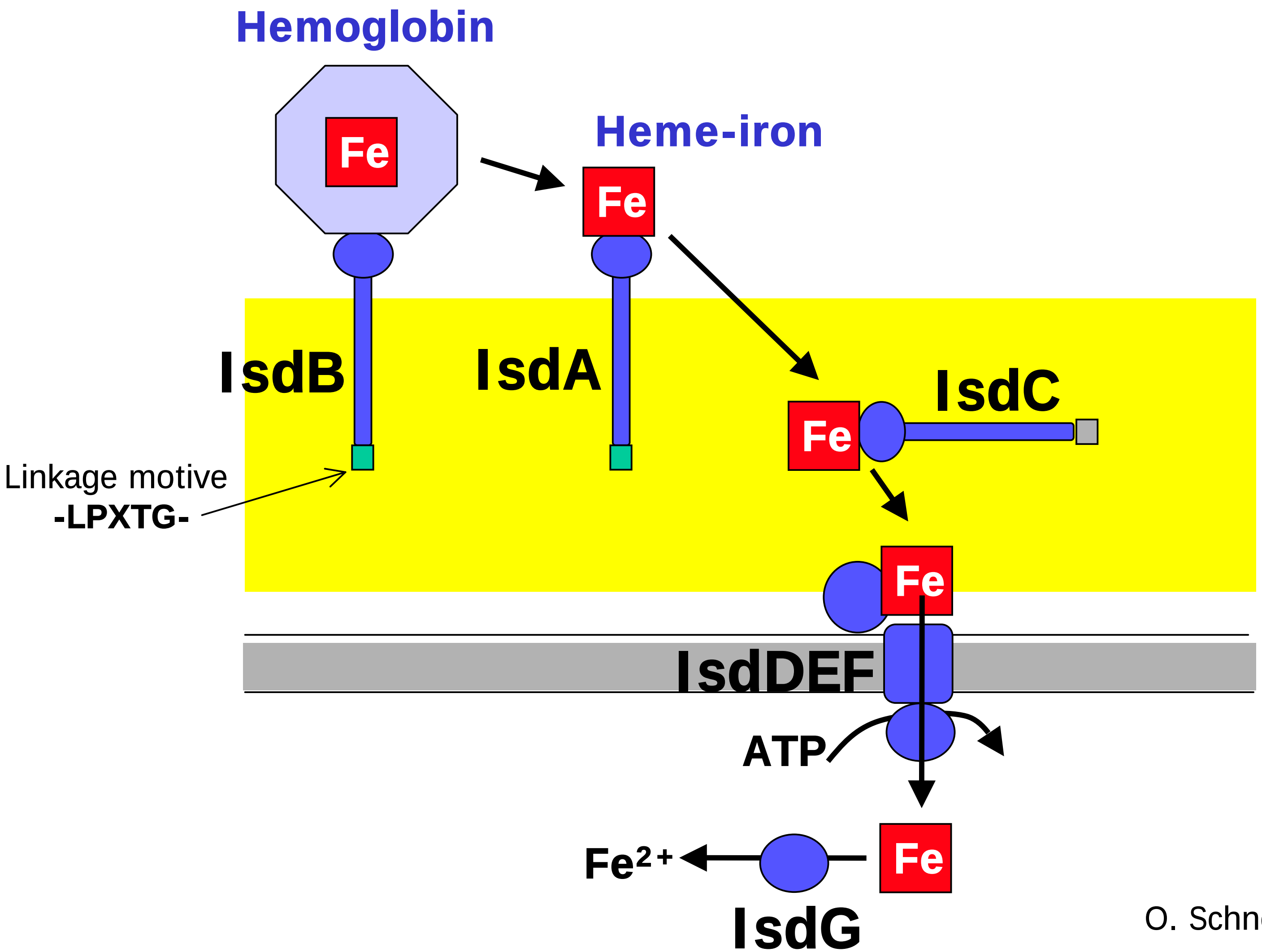


T.J. Foster *et al.*

Surface proteins with LPXTG motives:

- Binding proteins for fibronectin, fibrinogen, collagen,...
- Protein A (binding of IgG, van Willebrand factor, TNFαR
- Clumping factor (coagulation)

Putative pathway of heme-iron acquisition

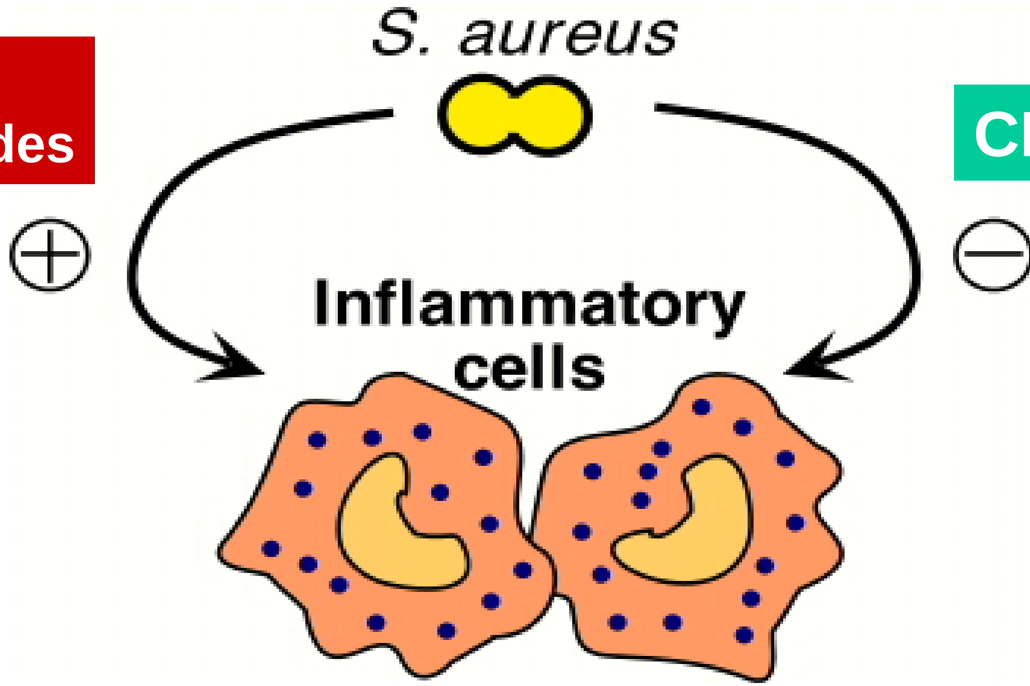


O. Schneewind *et al.*

Homologs of *isd* genes in: *Staphylococcus epidermidis*
Listeria monocytogenes
Bacillus anthracis

The pro- and antichemotactic *S. aureus* system

δ Toxin,
PSM peptides



CHIPS

M. Otto *et al.*

J. Van Strijp *et al.*

Leukocyte attraction: → **Release of nutrients**

Inhibition of chemotaxis: → **Establishing infection**

Conclusions:

S. aureus **is an extremely versatile human pathogen**

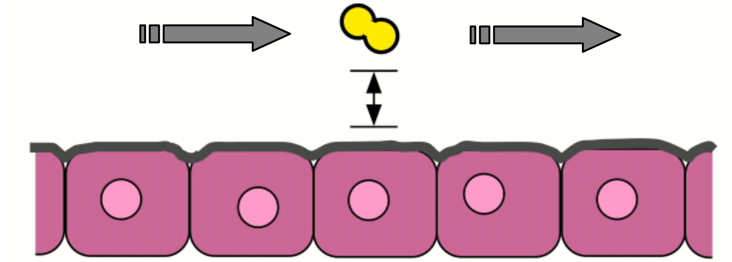
Many virulence factors are redundant

Several virulance factors have opposing activities

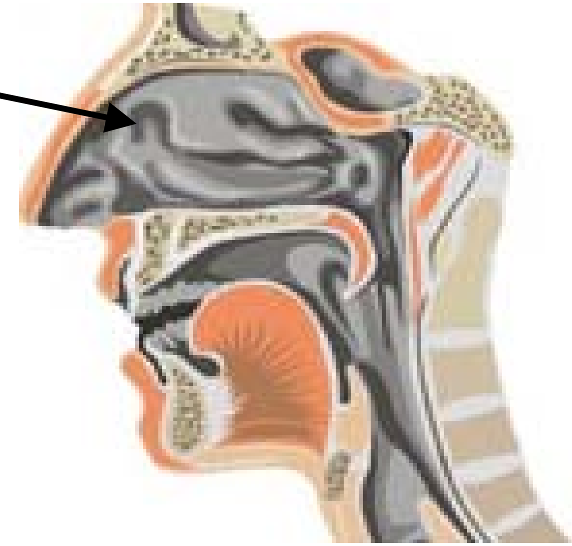
Complex regulation of virulence factors in response to environmental signals and bacterial density

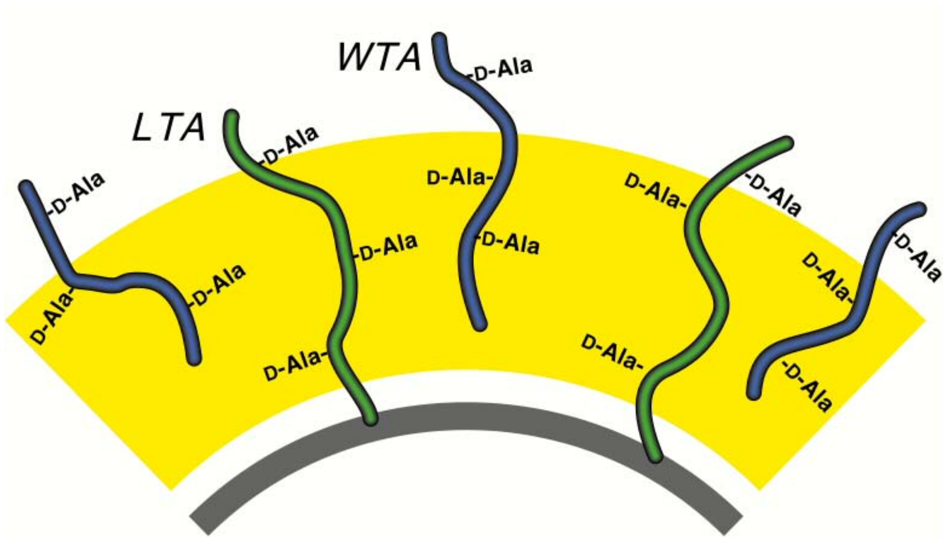
Molecular basis of *S. aureus* nasal colonization

Staphylococcus aureus



Nasal epithelial cells

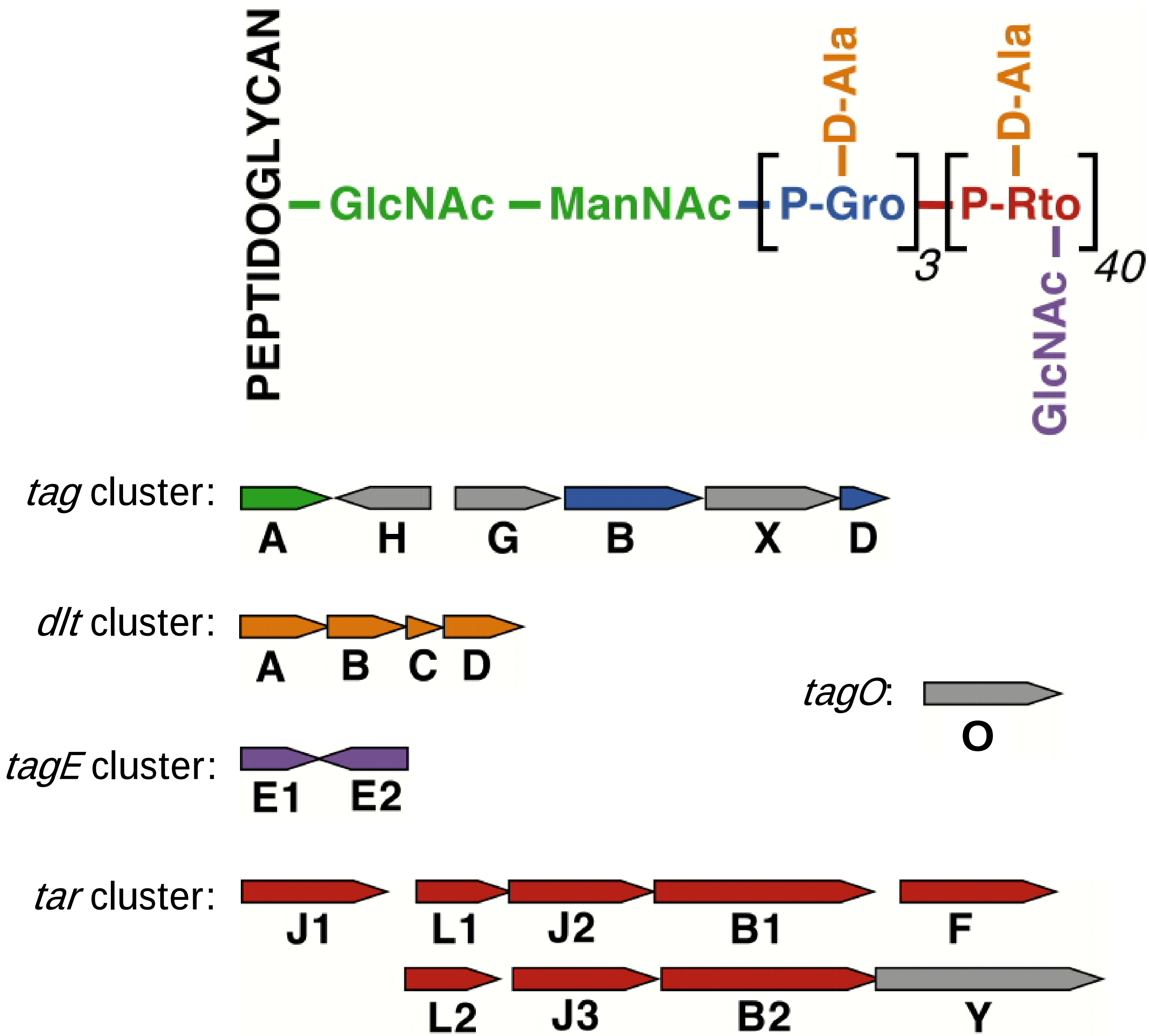




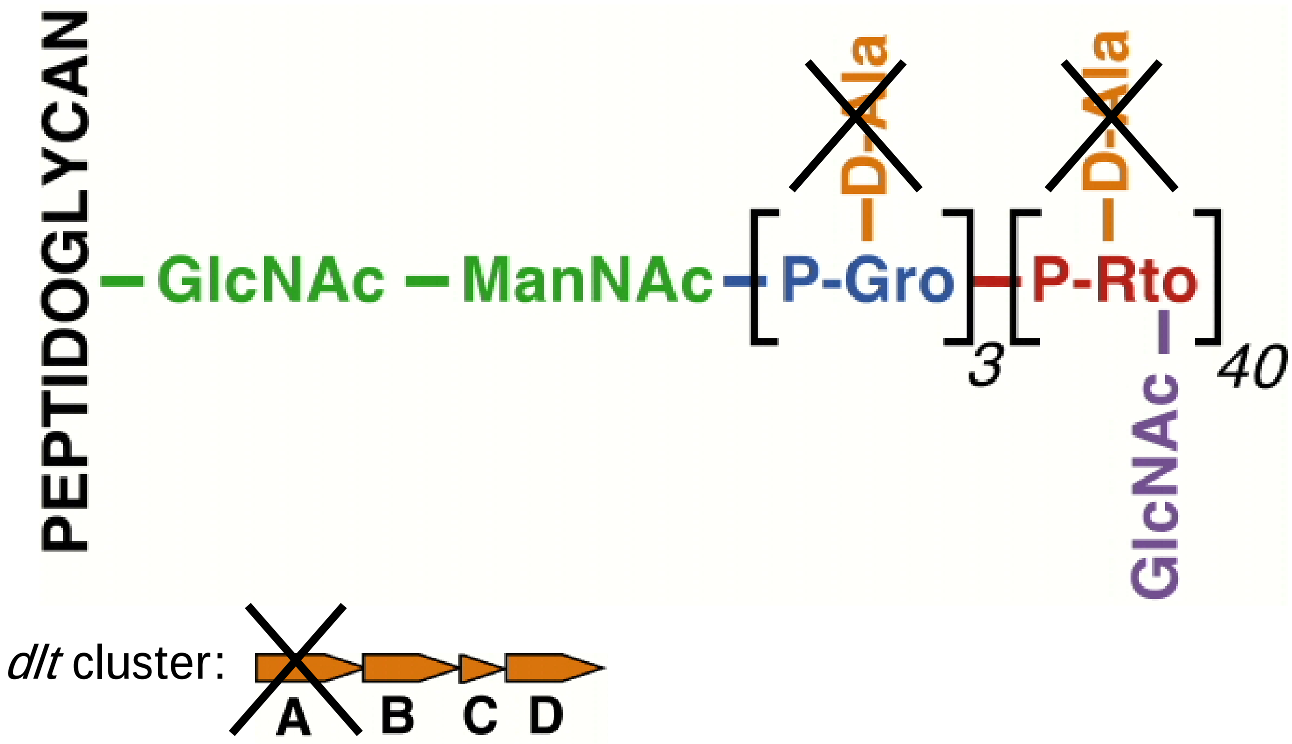
Role of keratin-binding proteins (ClfB)?

Role of teichoic acids?

>20 putative WTA genes in *S. aureus*



Inactivation of *dltA* in *S. aureus*



Mutant properties:

- WTA and LTA are **devoid of D-alanine**
- Susceptible to cationic antimicrobial peptides (Defensins, cathelicidins,...)

Peschel, A., et al. (1999) ***J. Biol. Chem.*** 274**:**8405

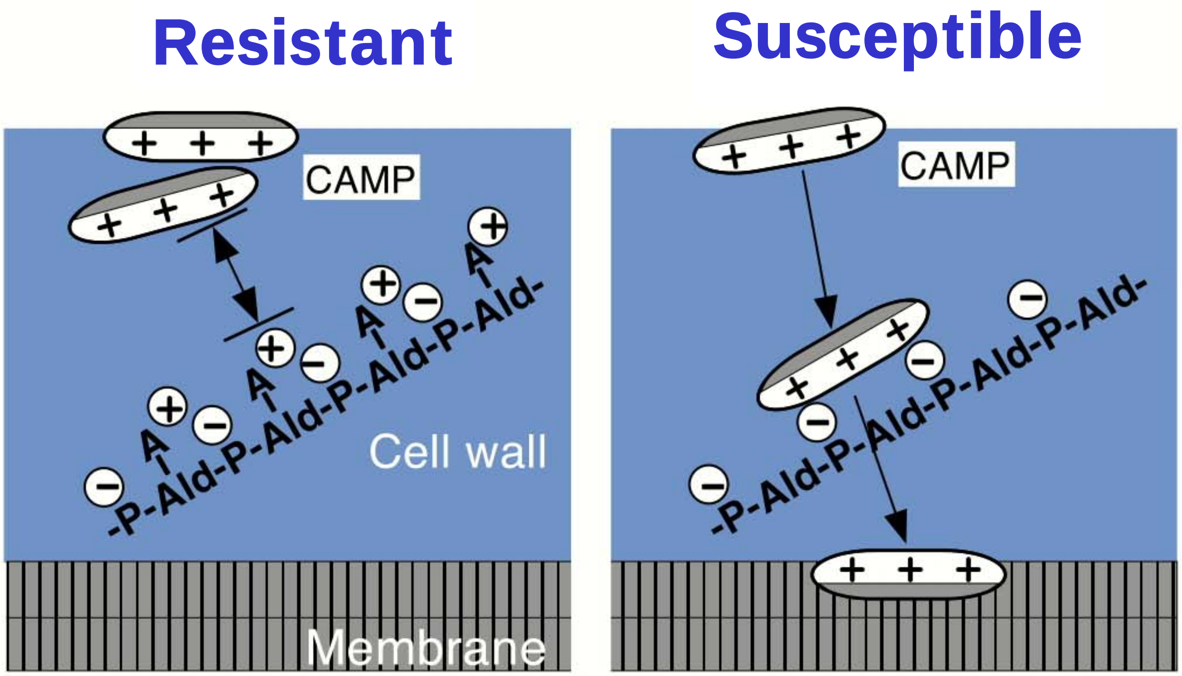
Teichoic acid D-alanine confers resistance to antimicrobial defensin peptides

Minimal inhibitory concentration of defensin hNP1-3:

S. aureus **wild-type**: **>60** µM

mutant Δ*dltA*: **2.9** µM

Resistance mechanism: Introduction of positive charges into the cell wall



Peschel, A. (2002) ***Trends Microbiol.*** 10, 179-186

Studying the role of WTA in nasal colonization?

Purified WTA inhibits *S. aureus* adherence to nasal epithelial cells

Aly R, Shinefield HR, Litz C, Maibach HI., *J. Infect. Dis.* **1980** 141:463

WTA is essential for viability of *Bacillus subtilis* and *Staphylococcus epidermidis*

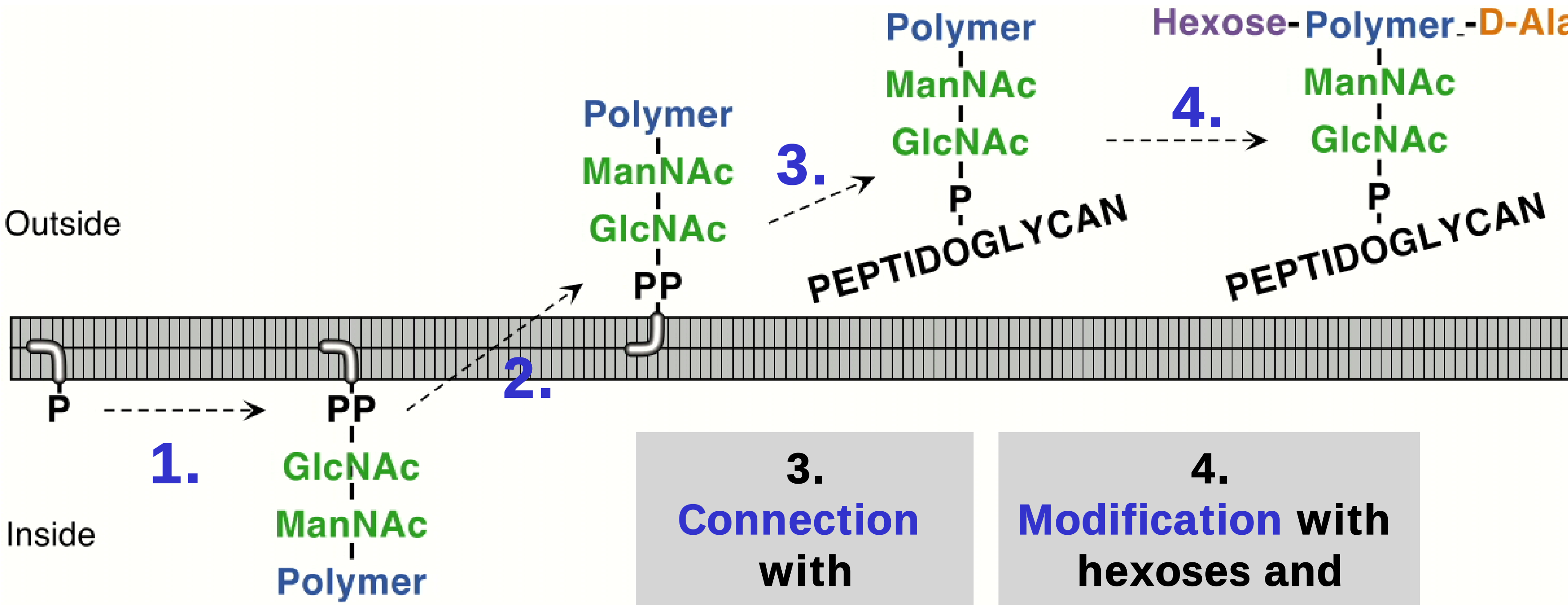
Soldo B, Lazarevic V, Karamata D., *Microbiology*. **2002** 148:2079

Fitzgerald SN, Foster TJ., *J Bacteriol.* **2000** 182:1046

Is WTA perhaps not essential in *S. aureus*?

Chatterjee AN. *J Bacteriol.* **1969** 98:519

Putative pathway of WTA biosynthesis



3.
Connection with peptidoglycan

4.
Modification with hexoses and D-alanin

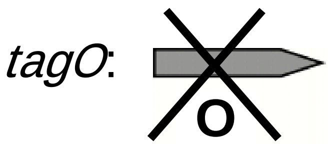
1.
Polymer synthese on Lipid-carrier

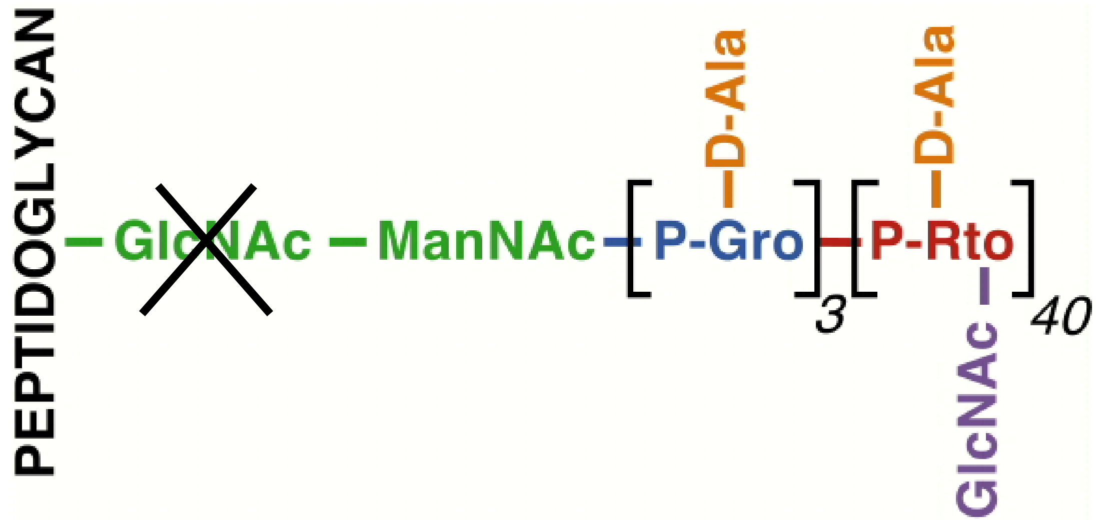
2.
Translocation

DltABCD

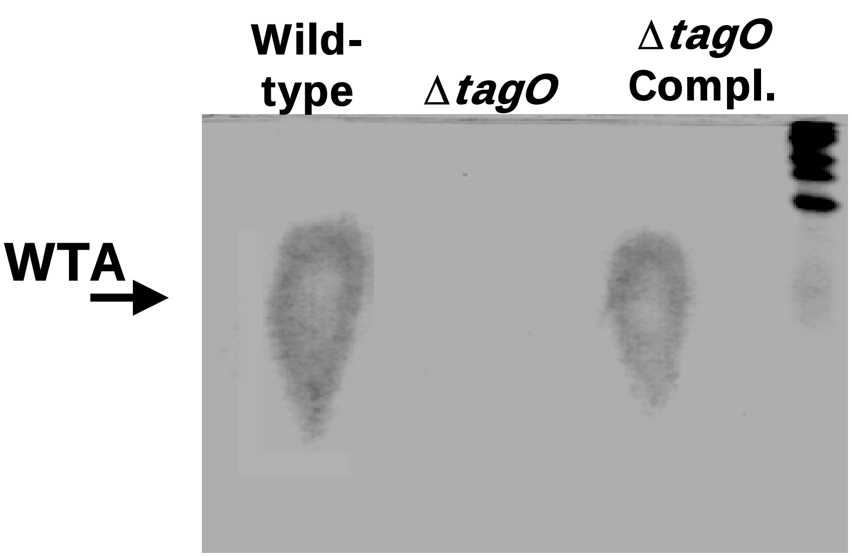
TagO?

WTA is not essential in *S. aureus*

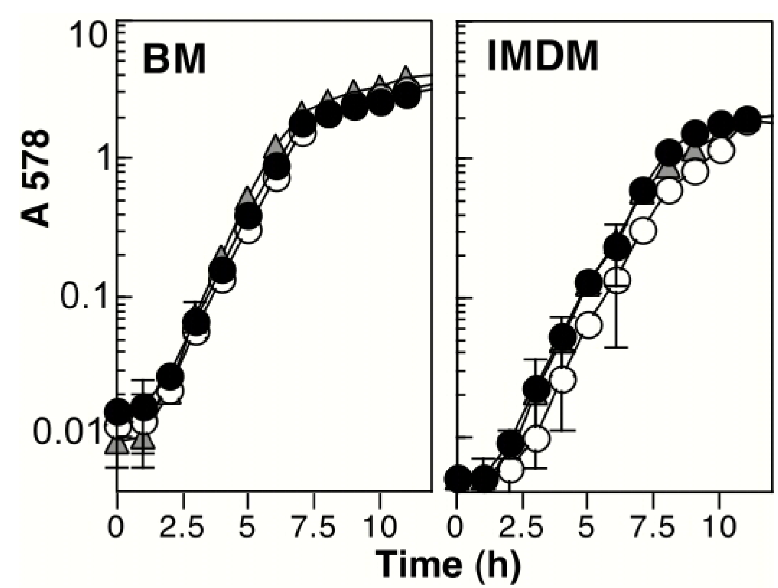




WTA-PAGE:

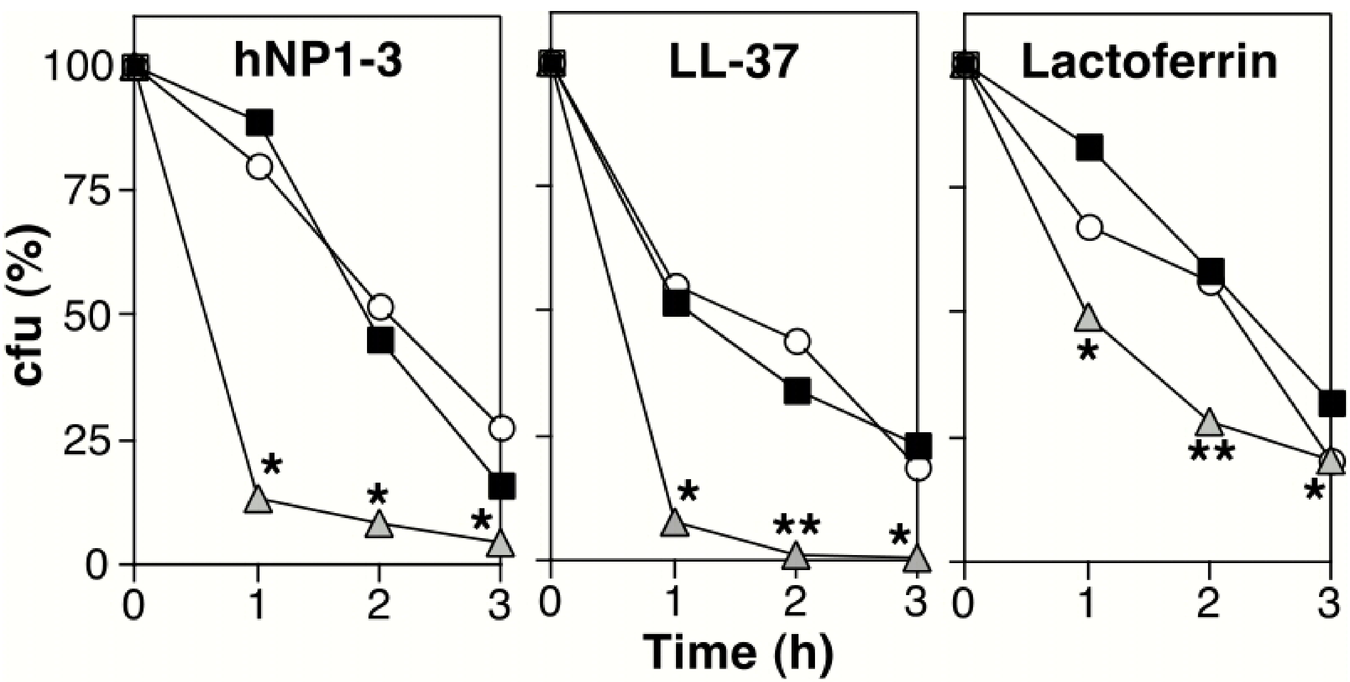


Growth kinetics:



No impact of WTA deficiency on susceptibility to CAMPs

Nasal antimicrobial peptides:



1. Wild-type
   - ➢ ***Normal WTA and LTA***
2. Δ*tagO*
   - ➢ ***No WTA***
3. Δ*dltA*
   - ➢ ***No D-Ala in WTA and LTA***

Weidenmaier, C, et al,. (2004) ***Nat. Med.*** 10, 243

WTA is required for nasal colonization

Nasal colonization of cotton rats

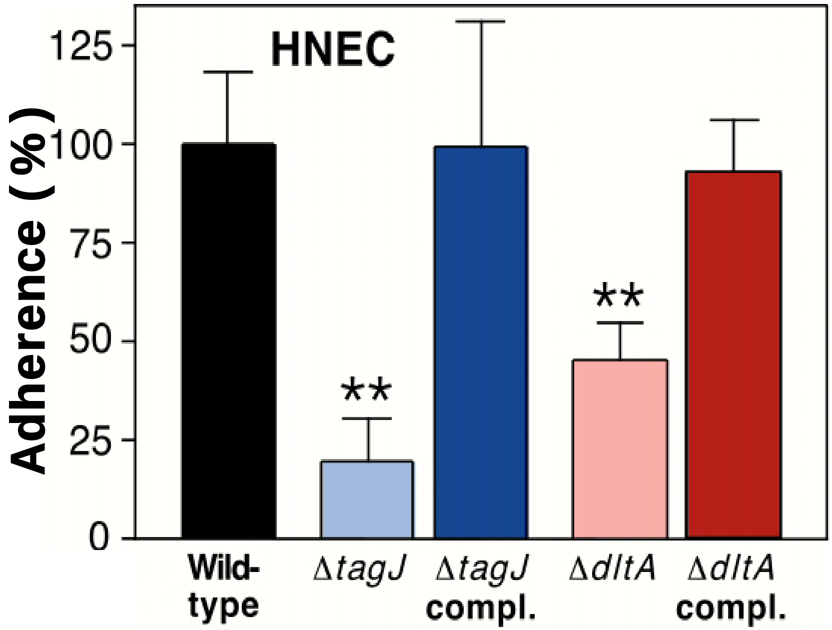
John Kokai-Kun et al., Gaithersburg

Wild-type: **15 / 15**

Δ*tagO:* (no WTA) **0 / 15**

Δ*dltA:* (no D-ala) **4 / 15**

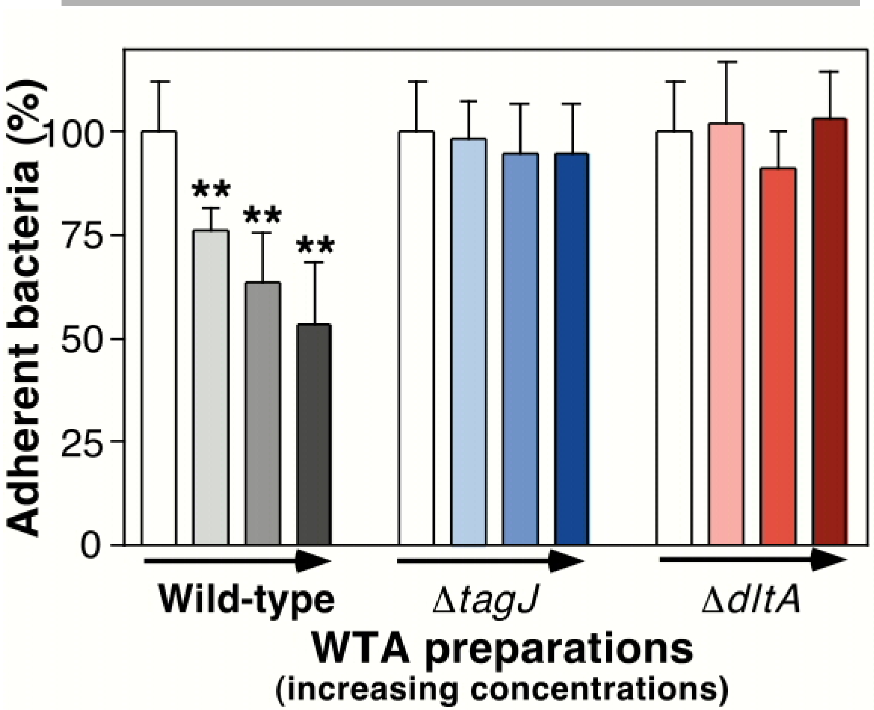
Adherence to human nasal epithelial cells



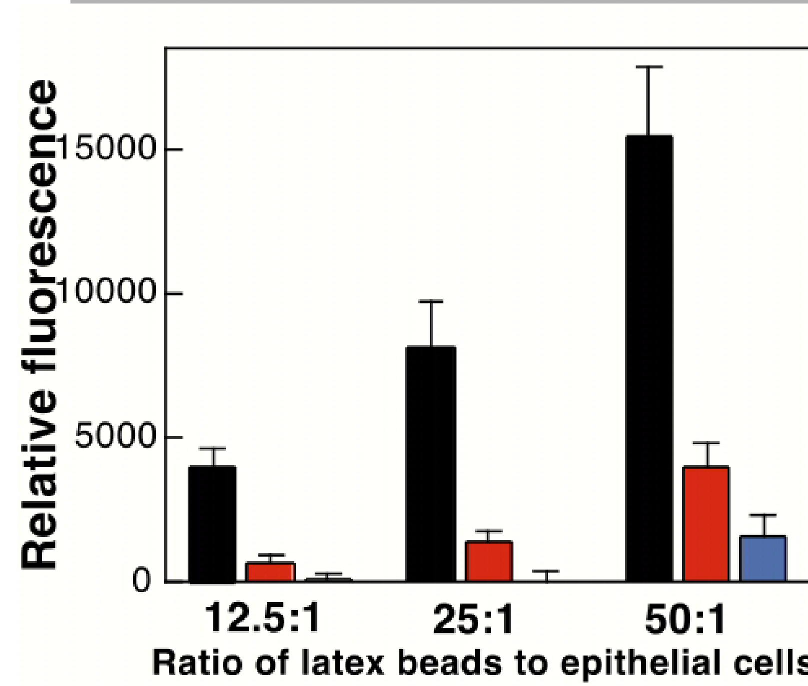
Weidenmaier, C, et al,. (2004) ***Nat. Med.*** 10, 243

WTA mediates specific attachment to human airway cells

Preincubation of A549 with WTA preparations



Binding of WTA-coated latex beads to A549



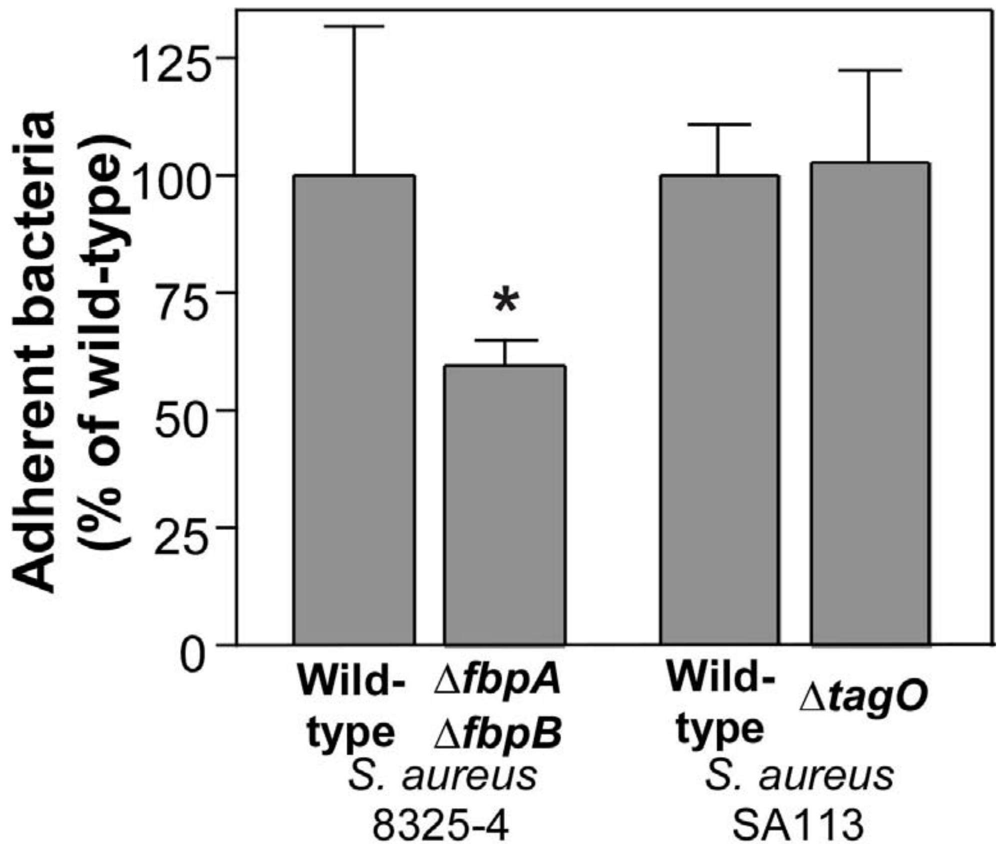
Wild-type
- ***Normal WTA and LTA***

Δ*tagO*
- ***No WTA***

Δ*dltA*
- ***No D-Ala in WTA and LTA***

WTA does not affect *S. aureus* binding to immobilized fibronectin

Adherence to fibronectin-coated microtiter plates

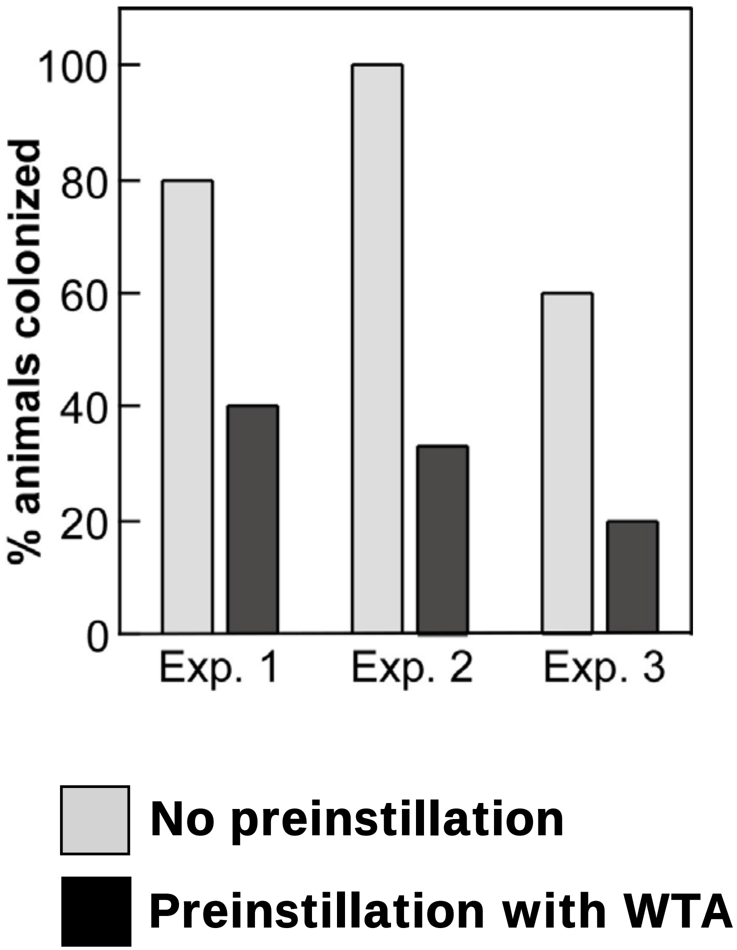


Similar results for other matrix proteins and keratin

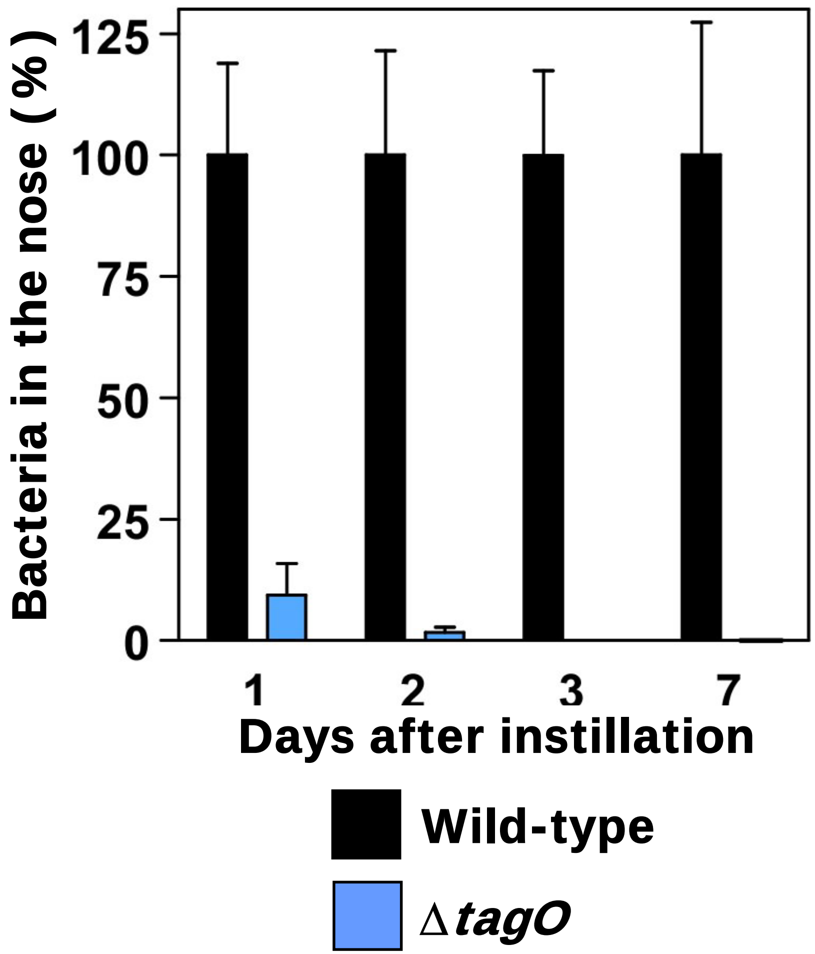
WTA mediates specific attachment in cotton rat noses

John Kokai-Kun et al., Gaithersburg

Preinstillation of WTA into cotton rat noses

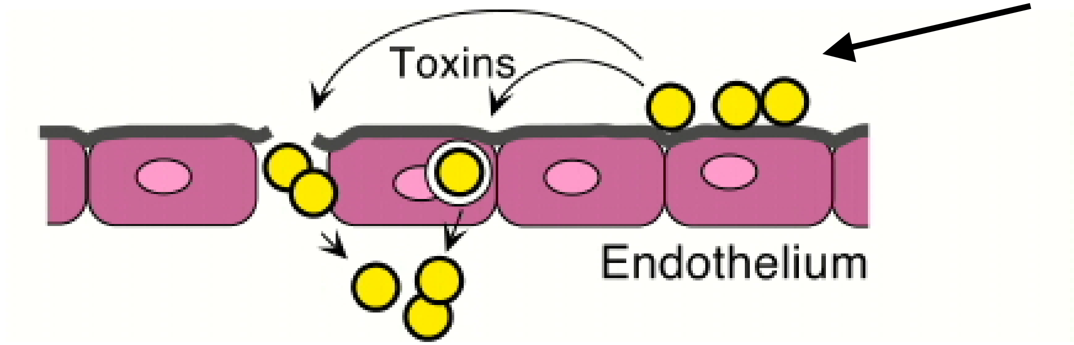


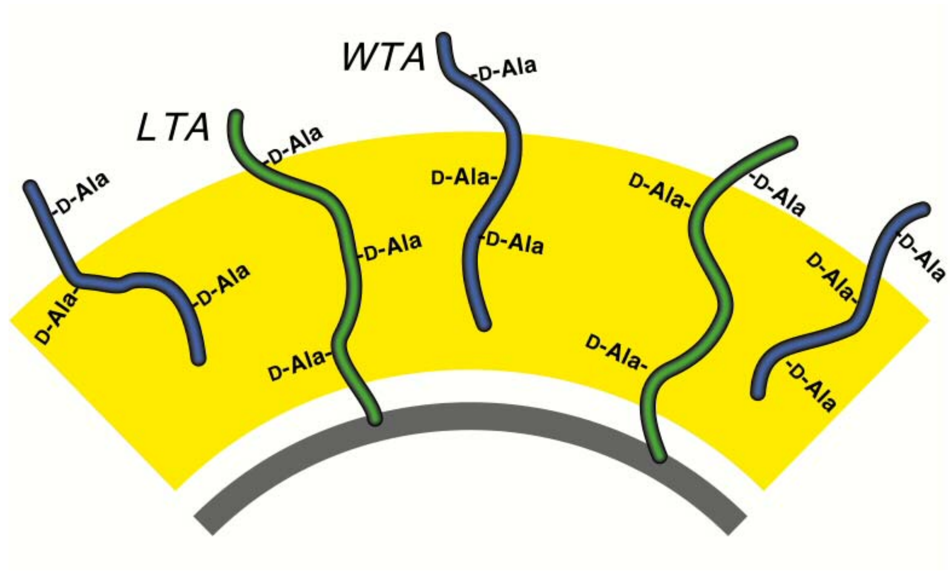
Kinetics of nasal colonization



S. aureus interaction with the endothelium

Staphylococcus aureus



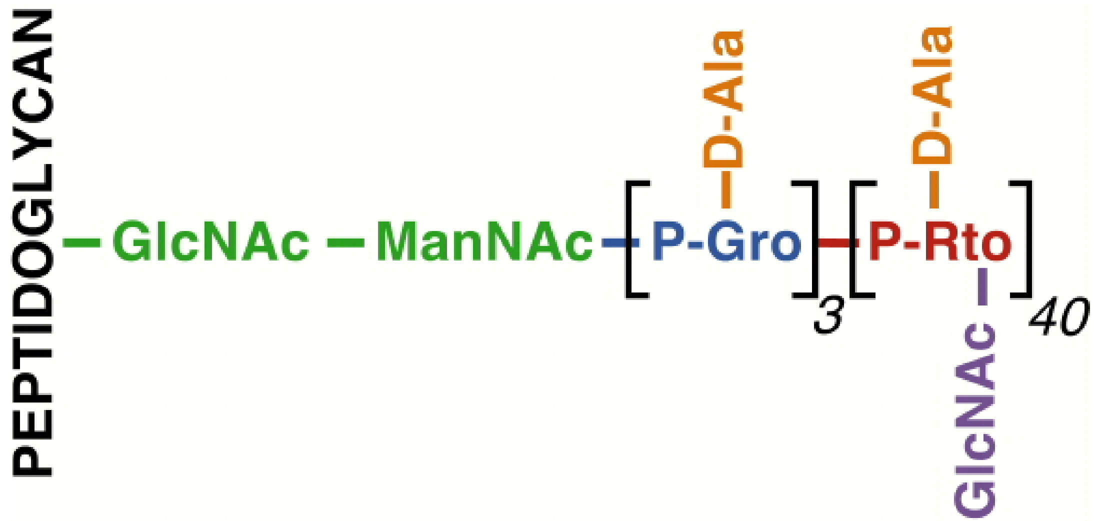


Role of fibronectin-binding proteins (FnbPs?)

Role of teichoic acids?

WTA structure is very variable

Staphylococcus aureus:



	Backbone:	Modification:
Staphylococcus aureus	Ribitol-P	D-Ala, GlcNAc
Staphylococcus aureus Type IV	Glycerol-P	D-Ala, GalNAc
Staphylococcus epidermidis	Glycerol-P	D-Ala, GlcNAc, Glc
Staphylococcus auricularis	GlcNAc-P	D-Ala, ?
Staphylococcus saprophyticus	Rto-P & Gro-P	D-Ala, GlcNAc
Listeria monocytogenes	Ribitol-P	D-Ala, GlcNAc / Gal / Glc
Bacillus subtilis	Glycerol-P	D-Ala, Glc

Conclusions:

WTA is not essential in *S. aureus*

WTA is required for nasal colonization and adherence to nasal epithelial cells

Involvement of WTA in host/skin tropism?

WTA is crucial for interaction with endothelial cells

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