The secret weapon that allows *Staphylococcus aureus* to hijack your cell cycle

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Host cells- Pathogens interactions

How do pathogens affect host cell pathways?

Host cells

- Promotion/inhibition of proliferation
- Alteration of apoptosis
- Cell cycle modulation
- DNA degradation/reparation

Berkova N et al., 2005; Femenia F, 2009; Oswald E, 2004, Sugai 1990
DNA damage during infection

DNA repair | Cell cycle arrest | Apoptosis

Level of DNA damage
The cell cycle of eukaryotic cells

The eukaryotic cell cycle involves:
- DNA replication
- Chromosomes segregation
- Cell division

FACS

G0/G1

S

G2/M

2n

4n

DNA Content (PI)
Cyclomodulins are bacterial effectors that interfere with the eukaryotic cell cycle.

<table>
<thead>
<tr>
<th>Effectors</th>
<th>Subunits/Properties</th>
<th>Phases Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxic</td>
<td>Three globular subunits</td>
<td>E.coli</td>
</tr>
<tr>
<td>Distending Toxin (CDT)</td>
<td>E.coli catalytic subunit CdtA, CdtB subunit Dnase</td>
<td>G1/S G2/M</td>
</tr>
<tr>
<td>H. hepaticus</td>
<td>CdtB catalytic subunit CdtB cell binding subunits</td>
<td></td>
</tr>
<tr>
<td>S. enterica serovar</td>
<td>CdtB subunit: Dnase and phosphatase</td>
<td></td>
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<tr>
<td>Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiga toxin (Stx) (Verotoxin)</td>
<td>AB5 enzymatic subunit StxA binding subunit</td>
<td></td>
</tr>
<tr>
<td>S. dysenteriae E.Coli (STEC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtilase AB (SubAB)</td>
<td>A subunit: N-glycosidase</td>
<td>S</td>
</tr>
<tr>
<td>AB5 toxin</td>
<td>E. coli (STEC)</td>
<td></td>
</tr>
<tr>
<td>A subunit: protease</td>
<td></td>
<td>G1/S</td>
</tr>
<tr>
<td>Anthrax toxin (Edema toxin/Lethal toxin)</td>
<td>SubA enzymatic subunit SubB binding subunit</td>
<td></td>
</tr>
<tr>
<td>Tripartite toxin</td>
<td>B. anthracis Edema and/or Lethal factor (A enzymatic subunit) Protective Antigen (B binding subunit)</td>
<td>G1/S</td>
</tr>
<tr>
<td></td>
<td>Edema factor: adenylate cyclase Lethal factor: zinc metalloprotease</td>
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</tbody>
</table>

Heterogeneous Family of Cyclomodulins: Smart Weapons That Allow Bacteria to Hijack the Eukaryotic Cell Cycle and Promote Infections.

Staphylococcus aureus-induced pathologies

Human: a wide range of clinical presentations

- Staphylococcus aureus
- Gram-positive bacterium
- is carried asymptomatically by up to 50% of healthy people
- In Europe, methicillin-resistant strains affect 150,000 patients annually
- The extra costs=380 millions €

Dairy cattle: mastitis
The cost: 78 € par animal /1 year
Does *S. aureus* produce **cyclomodulins**? What is the biological significance of the cyclomodulin action?
Cytopathic effect
Enlargement of the cells exposed to S. aureus.

MAC-T, cells produced from primary bovine mammary alveolar cells
HeLa, human epithelial cells from a fatal cervical carcinoma
**S. aureus** induces a G2/M phase delay

Heat-killed *S. aureus* or Latex beads don't induce a cell cycle arrest
Drop in the mitotic index in *S. aureus*-infected cells

Control
uninfected synchr

Synchronous cells

+ *S. aureus*
MW2 MOI 5:1 10:1 20:1

Uninfected synchr

MW2 MOI 5:1 10:1 20:1

Number of cells in metaphase or anaphase/telophase
total number of cells \( \times 100\% \)

Uninfect control

Synchr cells

+ *S. aureus MW2*

Mitotic Index

Uninfect cells control

+ S. aureus

Synchr

O46 MOI 5:1 10:1

Mitotic Index

0 10 20

asyn syn MOI 5:1 10:1

asyn syn MOI 5:1 10:1

* * ** **
Cyclins and *cyclin-dependent kinases* are 2 classes of regulatory molecules.

Cyclins control the cell cycle progression by activating *cyclin-dependent kinase*.

Dephosphorylation of Cdk1 at the late G2 phase activates Cdk1/cyclinB1 complex and triggers *mitotic* entry.
S. aureus-induced accumulation of phosphorylated Cdk1

Western blot

32 kD p-Cdk1

42 kD β-actin

Control   5:1     10:1    20:1
S. aureus MOI

Synchronous cells

Fold of control

Control   5:1     10:1    20:1
S. aureus MOI

18h

Control cells

G2/M 20%
S 7%
G1 73%

Cells + S. aureus

12
Histones package chromosomal DNA into nucleosome, the repeating unit of the chromatin.

Phosphorylation of Histone H3 at Ser10 is associated with the condensation of chromosomes during mitosis.
Western blot

17 kD p-H3S10
17 kD H3
42 kD β-actin

Control 5:1 10:1 20:1
S. aureus MOI

G2/M 20%
S 7%
G1 73%

18h Control cells

S. aureus induces a Ser10 dephosphorylation of histone H3

S. aureus-induced dephosphorylation of histone H3 Ser10

Synchronous cells

HeLa

Fold of

Control 5:1 10:1 20:1
S. aureus MOI

G2/M 41%
S 6%
G1 13%
Decrease in the number of p-Ser10 Histone H3-positive nuclei in *S. aureus*-infected cells
Gel-filtration chromatography of *S. aureus* supernatans

Bacterial cells and *S. aureus*-produced soluble factor induce the similar G2/M transition delay

Fractions 23-25 induce G2/M transition delay
<table>
<thead>
<tr>
<th>Uniprot Entry</th>
<th>Gene Name</th>
<th>Description</th>
<th>Peptide sequence</th>
<th>X!Tandem e-value</th>
<th>SEC Fractions identification</th>
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<tbody>
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<td>PSMA1_STAAW</td>
<td>psmA1</td>
<td>Phenol-soluble modulin alpha 1</td>
<td>GIIKVIKS</td>
<td>1,7E-3</td>
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<td>23, 24</td>
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<td>23, 24</td>
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<td>Phenol-soluble modulin alpha 3</td>
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<td>psmA3</td>
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<td>FVAKLKF</td>
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<td>23</td>
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<td>IIDIFAK</td>
<td>8,2E-3</td>
<td>24</td>
</tr>
</tbody>
</table>

Detection of PSMα peptide derivatives in *S. aureus* chromatography fractions.
Phenol-soluble modulins peptides (PSMs) define the virulence potential of *S. aureus*

- **PSMα1-PSMα4** are encoded in the psmα operon
- **PSMβ1 and PSMβ2** are encoded in the psmβ operon
- **δ-toxin** is encoded within the coding sequence for RNAIII, the RNA effector molecule of the accessory gene regulator (AGR) quorum-sensing system
Overview of phenol-soluble modulin activities
PSMα1 and PSMα2 induced the G2/M phases transition delay

Isogenic USA300 mutant lacking the PSMα operon does not induce G2/M phase transition delay
Internalized LAC wt replicated inside HeLa cells in contrast to its isogenic LACΔpsmaα mutant.

MOI 100:1

Log CFU/103 HeLa cells

2h 6h

Cells+ LACwt MOI Cells+ LACΔpsma Cells+ LACwt MOI Cells+ LACΔpsma

*
The G2/M transition delay results in the increase of internalization and intracellular bacterial replication.
Increase of mRNA levels of HBD-3 and -9 in PSMα1-treated cells
S. aureus-induced G2/M transition delay is strain-dependent
Staphylococcus aureus-Induced G2/M Phase Transition Delay in Host Epithelial Cells Increases Bacterial Infective Efficiency

Ludmila Alekseeva¹,²,³,⁹, Lucie Rault²,³,⁹, Sintia Almeida²,³,⁴, Patrick Legembre⁵, Valérie Edmond⁵, Vasco Azevedo⁴, Anderson Miyoshi⁴, Sergine Even²,³, Frédéric Taieb⁶, Yannick Arlot-Bonnemains⁷, Yves Le Loir²,³, Nadia Berkova²,³,*

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Phenol-soluble modulin α induces G2/M phase transition delay in eukaryotic HeLa cells

Martine Deplanché,*⁺,¹ Rachid Aref El-Aouar Filho,*⁺⁺,¹ Ludmila Alekseeva,*⁺⁺,⁺⁺,⁸ Emilie Ladier,*⁺ Julien Jardin,*⁺ Gwénaële Henry,*⁺ Vasco Azevedo,⁺ Anderson Miyoshi,⁺ Laetitia Beraud,⁺ Frederic Laurent,⁺⁺GERARD LINA,⁺⁺François Vandenesch,⁺⁺⁺⁺ Jean-Paul Steghens,⁺⁺⁺⁺ Yves Le Loir,*⁺ Michael Otto,⁺⁺⁺⁺ Friedrich Götz,⁺⁺⁺⁺ and Nadia Berkova*⁺⁺,⁺⁺,²
Impact of S. aureus lipoproteins on the cell cycle progression

Structure and post-translational modifications of S. aureus lipoproteins
The *lpl* gene cluster of *S. aureus* is involved in the induction of the G2/M delay
Impact of S. aureus lipoproteins on the cell cycle progression

Protein portion of lpl plays a crucial role in S. aureus-induced G2/M delay.
Impact of S. aureus lipoproteins on the cell cycle progression

Structure of Pam3 of S. aureus lipoprotein

Fournier, 2013
The synthetic lipopeptides Pam2C and Pam3C extended the G2 phase DNA content.

Control synchronous cells

- G1: 30±4%
- S: 9±2%
- G2: 61±5%

Synchronous cells +Pam2 (5 µg)

- G1: 41±4%
- S: 11±3%
- G2: 48±5%

Synchronous cells +Pam3 (5 µg)

- G1: 39±3%
- S: 9±2%
- G2: 52±3%

Pam2 > Pam3

Pams are synthetic lipopetides that mimic the acylated amino terminus of bacterial lipoproteins

The protein part of *S. aureus* lipoproteins, a new cyclomodulin, plays a pivotal role in the G2/M transition delay.
Bacterial cyclomodulins alter the eukaryotic cell cycle for their own benefit

CONCLUSIONS

S. aureus PSMs and Ipl are new cyclomodulins, which

- subvert the host cell cycle progression
- increase a bacterial internalisation
- augment an intracellular bacterial proliferation
- inhibit a host protective response

This is a newly identified mechanisms of S. aureus strategy for fostering an infection
Participants/Collaborations


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MG university, Brazil
Sintia Almeida
Vasco Azevedo, Rachid Filho

Centre International de Recherche en Infectiologie, INSERM U1111, CNRS UMR5308, Université Lyon 1, Gerard Lina, Frederic Laurent

Hospices Civil de Lyon
Laetitia Beraud, Jean-Paul Steghens

University of Tübingen, Microbiology, Germany
Friedrich Goetz
Thank you for your attention