

Facteurs de virulence des bacilles à Gram négatif

B. Polack
GREPI - EA 2938
Université Joseph Fourier - Grenoble

1

Généralités

2

Interactions Hôte -Pathogène

- « Existing concepts of virulence and pathogenicity are inadequate because they do not account for the full complexity of microbial pathogenesis in host... »
- Given our incomplete knowledge of host-pathogen interaction, it should be considered as a work in progress ... »

Casadevall & Pirofski I&I 1999,67,3703

3

Interactions hôte-microorganisme

- Exposition
 - Infection
 - Colonisation
 - Commensalisme
 - Latence
 - Maladie
- } Définis par la nature et l'intensité des lésions de l'hôte

Casadevall & Pirofski TIM 2003,4,157

4

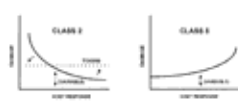
Modèles d'interactions

Pathogène si réponse immunitaire faible



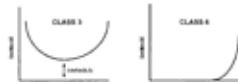
Pathogène quand réponse immunitaire faible ou forte

Pathogène si réponse immunitaire faible ou au début de la réponse imm.



Pathogène pendant la réponse immunitaire et si RI forte

Pathogène du début à la fin de la réponse immunitaire



Pathogène si et ss réponse immunitaire forte

Casadevall & Pirofski I&I 1999,67,3703

5

Exemples

TABLE 2. Examples of weak and strong responses that can be associated with host damage^a

Evaluation	Description of response	
	Weak	Strong
Quantitative	Insufficient number of immune effector cells and/or molecules to prevent host damage	Overproduction of inflammatory mediators that result in tissue fibrosis or promote malignant transformation
Qualitative	(i) Antibodies of specificities or isotype that do not mediate protection (ii) Th2 responses instead of Th1 responses for pathogens that require Th1 responses for containment ^b	(i) Antigenic mimicry (ii) Eosinophilic inflammation in response to certain antigens ^c (iii) Antibody-mediated enhancement of disease

^a The appropriateness of weak and strong responses must be considered in the context of specific pathogens.

^b Excessive inflammatory responses may be useful for helminths but not certain fungi.

^c Th2 responses are associated with strong antibody responses whereas Th1 responses are proinflammatory (15, 20). Th2 responses to pathogens that require strong cellular inflammatory responses for containment and eradication may result in chronic and progressive infection. However, it is noteworthy that this may be an oversimplification of a very complex process (1).

Casadevall & Pirofski I&I 1999,67,3703

6

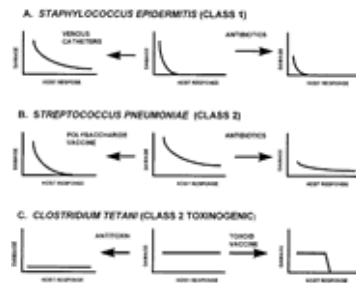
Exemples

classe	pathogène	Lésions en fonction de la réponse immune		
		faible	moyenne	forte
1	<i>P.carinii</i>	pneumonie	∅	∅
2	<i>B.anthraxis</i>	anthrax	anthrax	∅
3	CMV	pneumonie...	MNI	Guilain Barré
4	<i>Aspergillus spp</i>	aspergilose invasive	∅	sinusite allerg. pousm. Fermier
5	<i>Trypanosoma</i>	trypanosomiase	trypanosomiase	cardiomyopathie
6	<i>H.pylori</i>	∅	∅	ulcère, carcinome, lymphome

Casadevall & Pirofski I&I 1999,67,3703

7

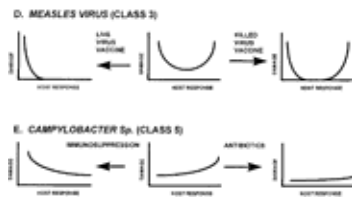
Effets du traitement



Casadevall & Pirofski I&I 1999,67,3703

8

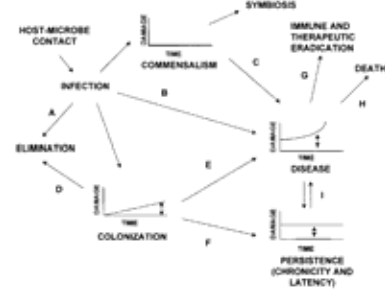
Effets du traitement



Casadevall & Pirofski I&I 1999,67,3703

9

Devenir de l'interaction hôte pathogène



Casadevall & Pirofski I&I 200,68,6511

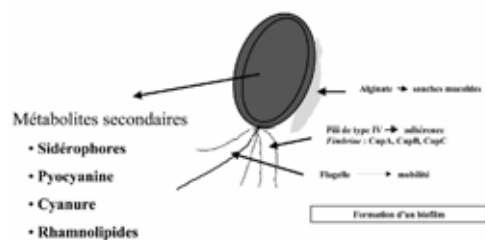
10

Pseudomonas aeruginosa

Un modèle de choix pour l'étude d'une bactérie pathogène opportuniste

11

Facteurs cellulaires de virulence



Lazdunski Ann Fr Anesth Réanim 2003,22,523

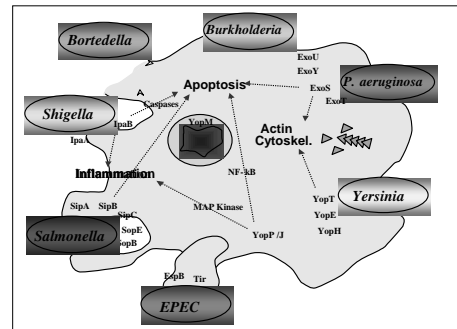
12

La cytotoxicité

Le Système de Sécrétion de Type III OU SSTT

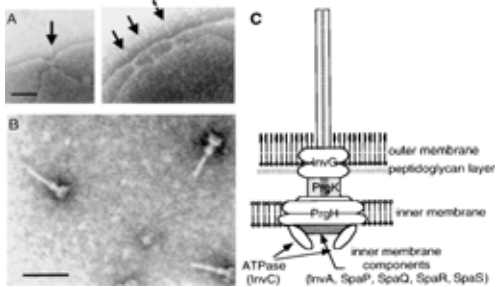
19

TTSS protein secretion in mammals



20

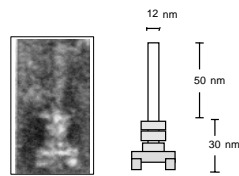
TTSS: a needle



Kubori et al. Science 1998;280:602

21

Size of the needle

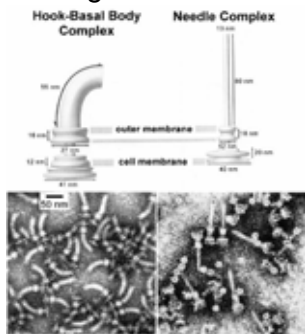


- Internal diameter:
 - 2 to 3 nm
- How proteins travel
 - unfolded ?
 - de-folded ?
 - chaperone ?

Kimbrough & Miller PNAS 2000;97:11008

22

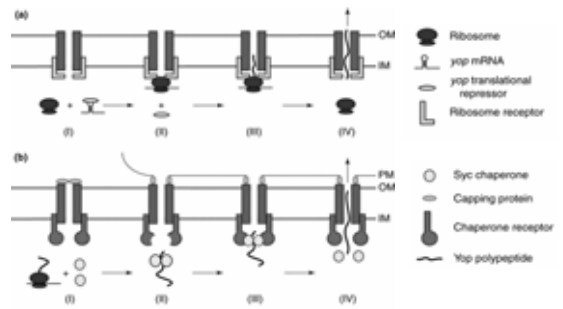
Flagelle et SSTT



Macnab Annu Rev Microbiol 2003;57:77

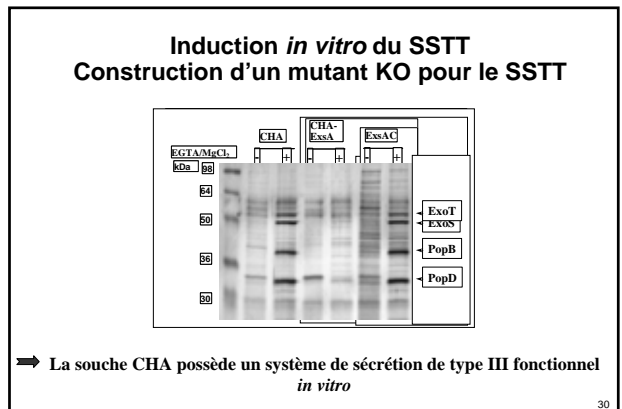
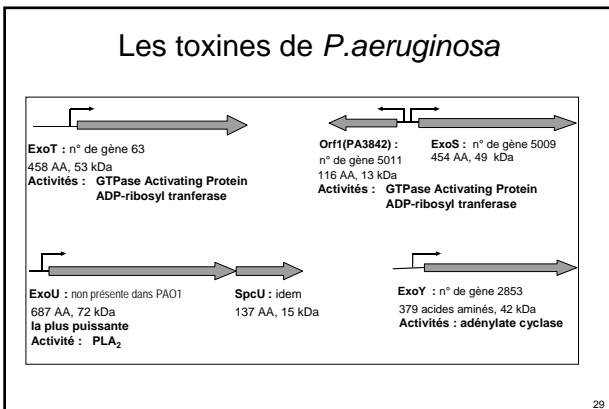
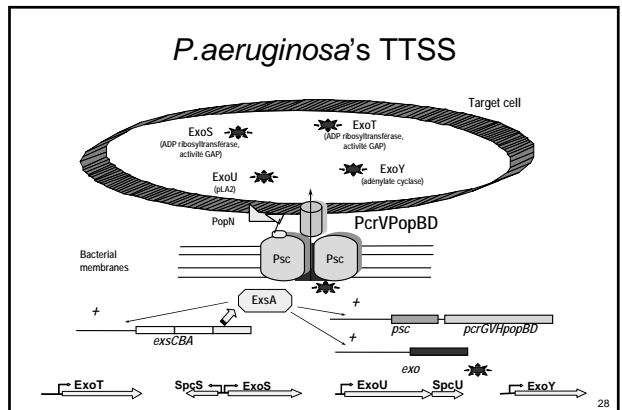
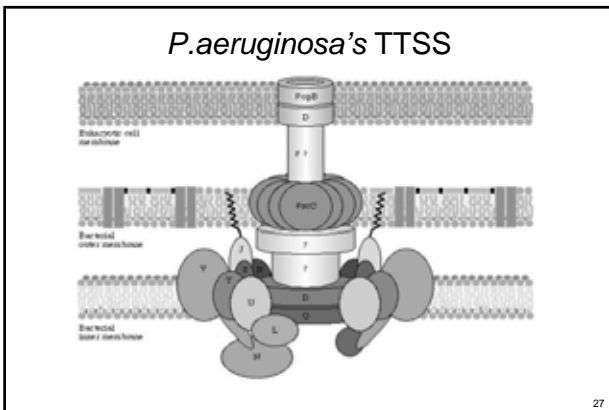
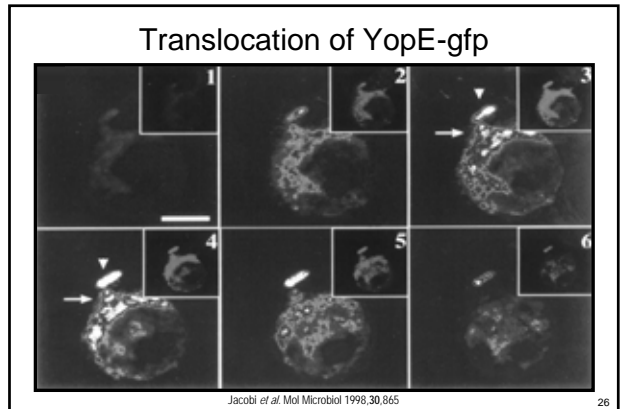
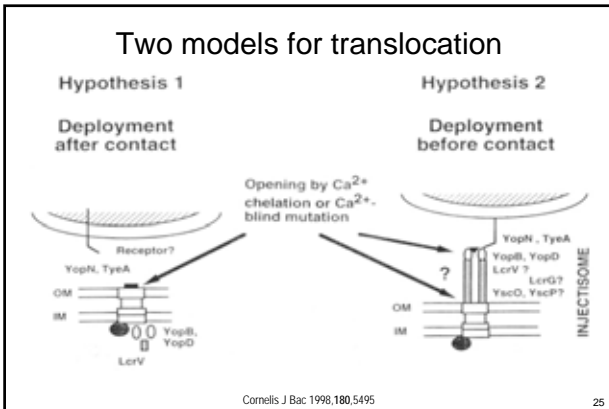
23

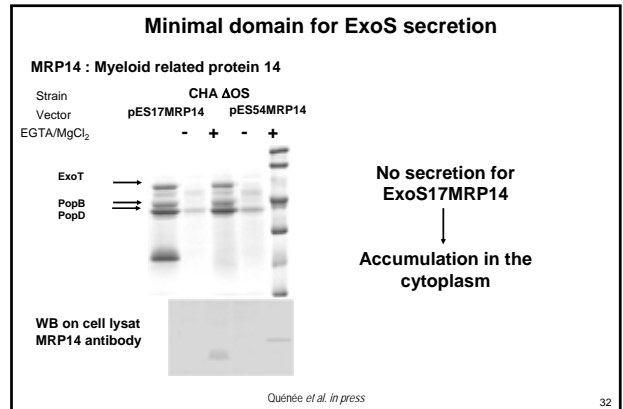
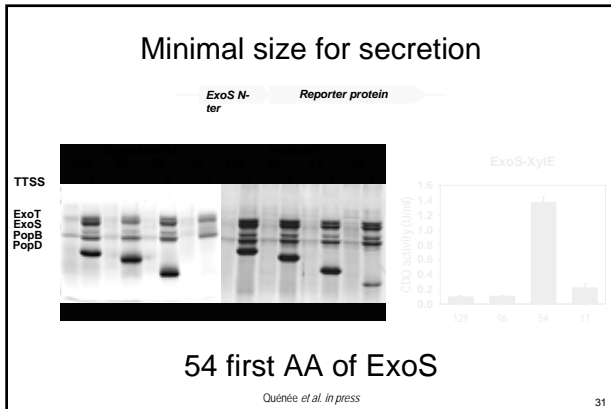
Two models for secretion



Cheng & Schneewind, Trends Microbiol. 2000;8:214

24



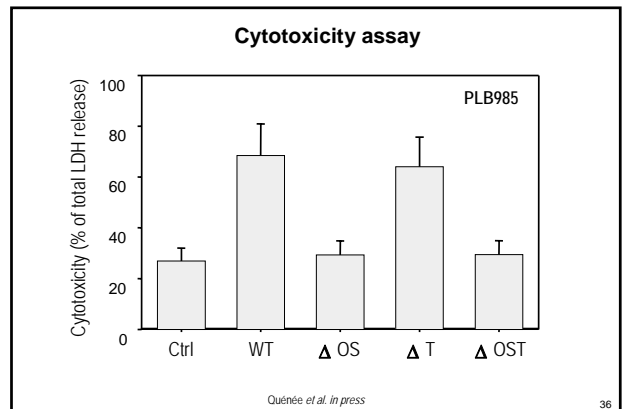
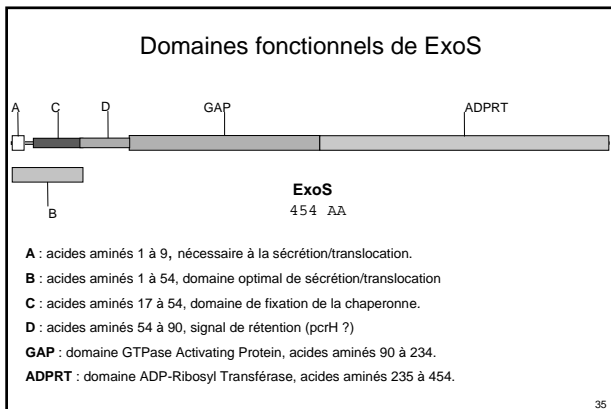
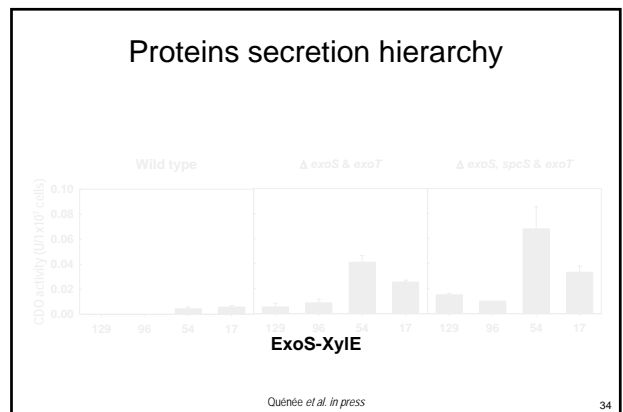


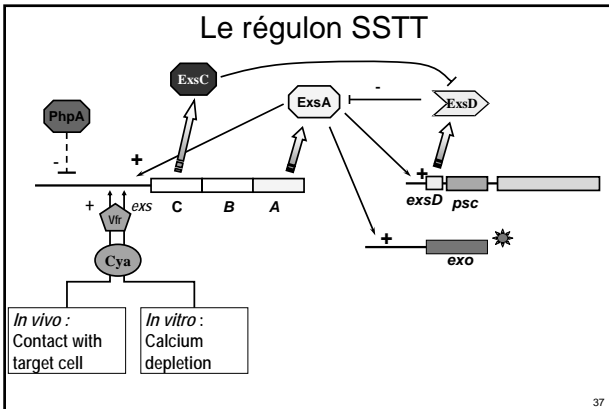
Minimal domain for ExoS secretion

	Secretion		Production	
	54 AA	17 AA	54 AA	17 AA
Luciferase	-	-	+++	+++
IVY	+++	+	nd	nd
CDO (<i>xylE</i>)	+++	+	+++	+
MRP14	+++	-	+++	+++

Secretion and production depend on the minimal domain and depend on reporter genes used

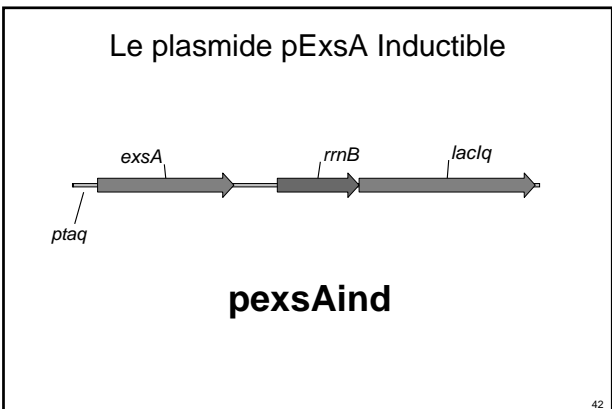
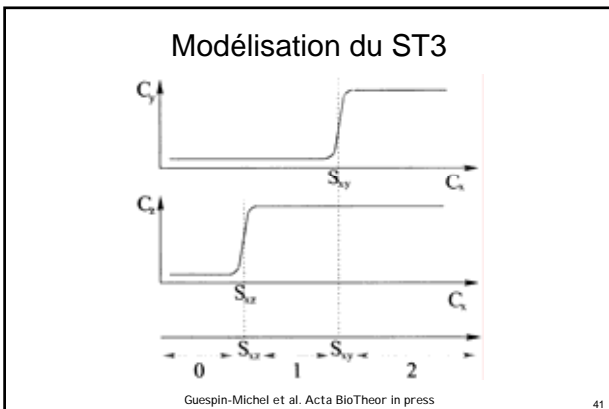
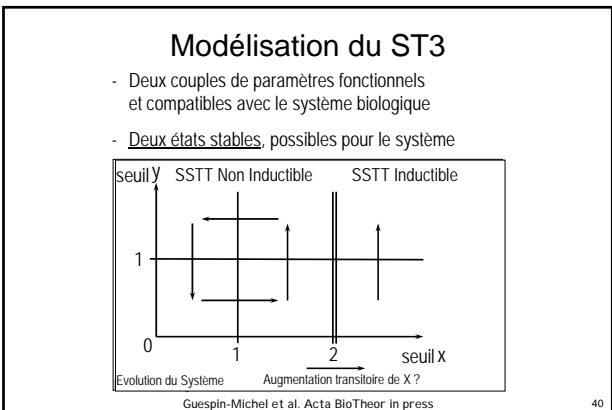
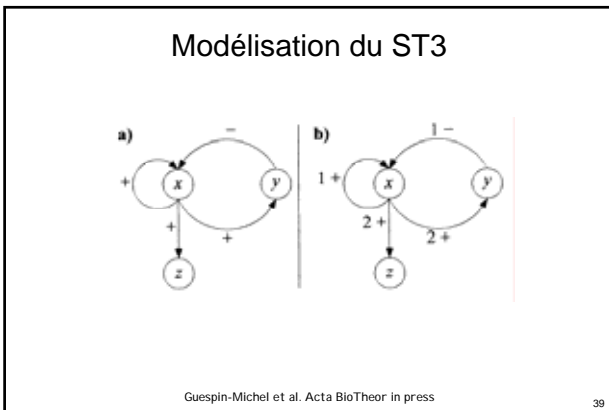
Quénée et al. in press 33



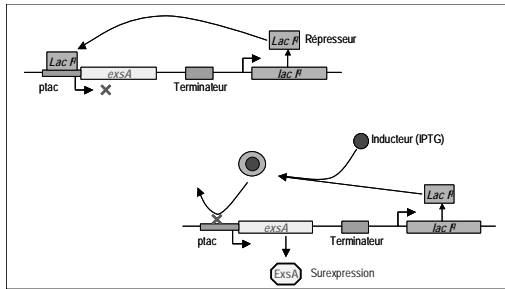


Phénotypes observés du SSTT

- Il existe trois états potentiels de ExsA
 - état 1 : pas d'induction possible
 - état 2 : le système est inducible
 - état 3 : le système est induit et peut sécréter



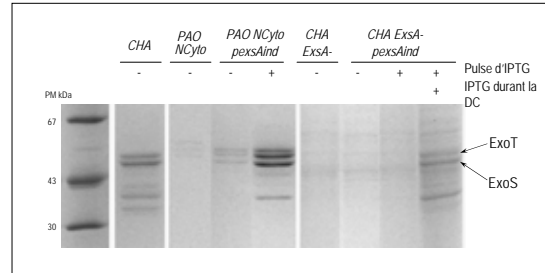
Système d'expression inductible



Contrôle de la surexpression d'ExsA

43

Pulse d'ExsA et sécrétion en déplétion calcique

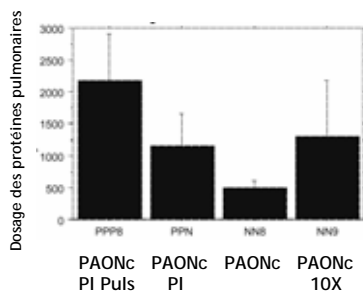


→ L'augmentation transitoire d'ExsA dans la souche PAONC la rend inductible en déplétion calcique ⇔ Passage d'un état à l'autre du système.

Filopon et al.

44

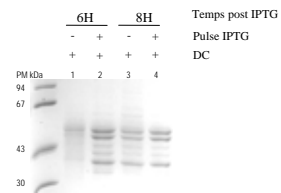
Effet *in vivo*



Filopon et al.

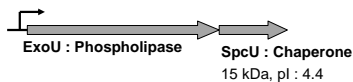
45

Limites actuelles



46

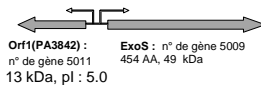
P.aeruginosa TTSS chaperone



(Frank D et al, J.Bact 1998)

SpcU bind ExoU in the N-terminal domain of the toxin
SpcU may function by keeping ExoU from aggregation in the cytoplasm
Absence of SpcU only reduce ExoU secretion

What about Orf1 ?



Orf1 (PA3842) : n° de gène 5011
13 kDa, pl : 5.0

ExoS : n° de gène 5009
454 AA, 49 kDa

47

Chaperone alignment

	β1	β2	β3
SicP (1)	EDRQDTEANRERKLCGLPLTFDNNCLDLEL	ESDFTSREAN	D-DIMENNG
SigE (1)	---MSSANRFLYALGQDAPSE--SFLDID	D-GLOVE	MES-D-RHFXCC
CesT (1)	SGSRKELLNFAKXGIGISFENKLCQIATKIVYVYKESANR	---	---
SycE (1)	MYEFOATDQEEQELSDPTIEPVKQW	---G	EFACHTREPV-GGELMPT
Orf1 (1)	MNPLYRAAEQEEALDLEPTPEKESV	---	PLCHLREKPTDR-ELMPT

	β4	β5
SicP (54)	ELPLPVCDDSIHQVVIHQELIANN-NGLVADAN	LEELHAT--DET
SigE (45)	FPFLP--DDILTLOHLELNYS---	AVTQZADSTLEVALYRL--QTE
CesT (54)	WCKPFTD-NPWFALFELNANLNFENQ-GPVL	WESGQSELEALRPT--LDA
SycE (54)	LDG---LDNNK--STLSEKSP--SGDLKPL	SEVQYKFWKHWKQWLE
Orf1 (52)	LLD---QDQATSEKQNFSPDPCF---	LDLHPKREKELMNRGQ-LLE

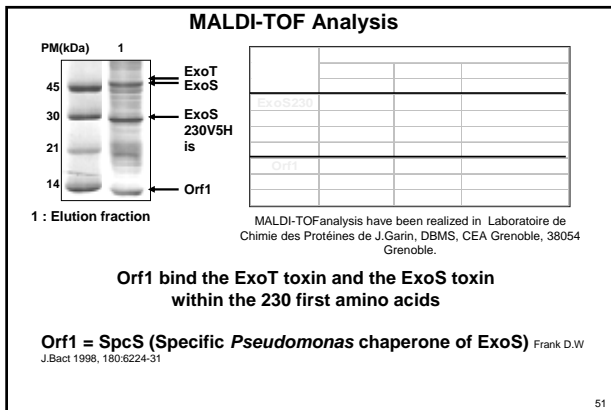
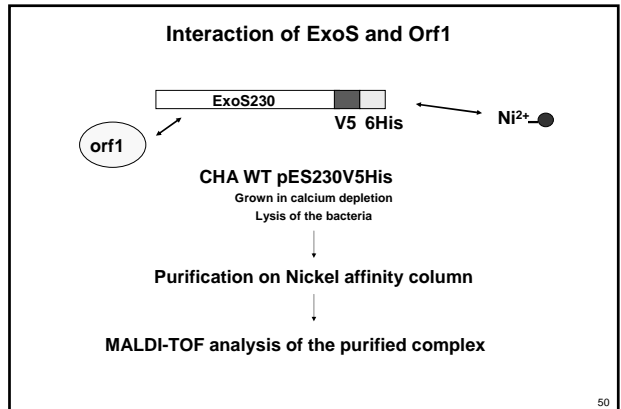
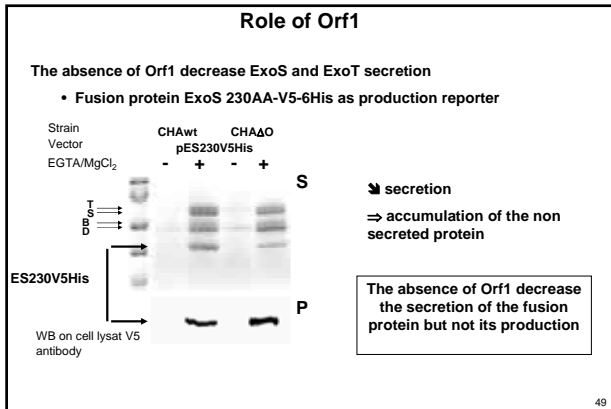


Chaperone may form dimer

Interaction with the N-terminal domain of type III toxins

Strebbsins & Galan, Nature 2003

48



What we know now

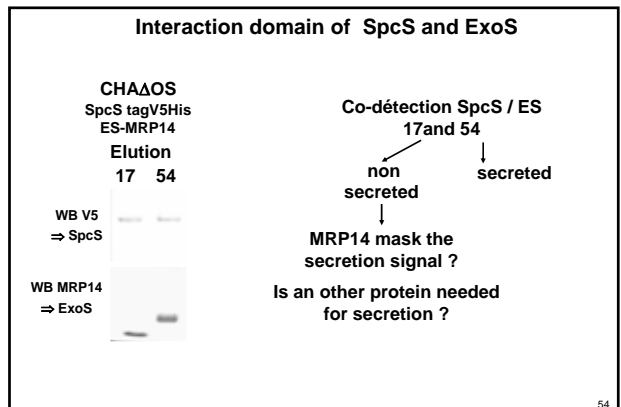
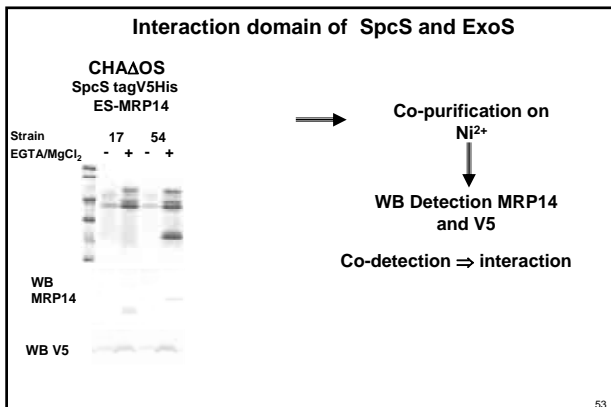
SpcS allows an optimal secretion

The 54 first amino acids are necessary for the optimal secretion/production

The 17 first amino acids are not sufficient for the optimal secretion/production

SpcS bind ExoS on the N-terminal domain
↪ Between amino acids 17 and 54 ?

52



CysB is an other member of the secretion complex

		CysB	
		CysB	

CysB : transcriptionnal factor (Toussaint B et al, Mol Micro 1997)

CysB + *algD* ⇒ biofilm formation

Is CysB participating in the switch off of cytotoxicity phenotype to biofilm formation ?

55

CHAΔCysB and CHAΔOΔCysB

Southern blot CysB probe

2000 pb
1500 pb

WT SR ΔC ΔOΔC DR

Strain EGTA/MgCl₂ WT ΔO ΔC ΔOΔC

ExoT ExoS
ES230V 5His

WB with V5 antibody

CysB invalidation ⇒ production/secretion

Double mutation Sps/CysB ⇒ secretion production

CysB : negative regulator ?

56

TTSS activation in CHAΔCysB

pC + *exs* C B A ⇒ ExsA

pC GFP Transcriptionnal fusion

Strain	EGTA/MgCl ₂	% relative fluorescence
CHA WT	-	~25
CHA WT	+	~100
CHA ΔCysB	-	~50
CHA ΔCysB	+	~250

57

Role of CysB

CysB + *algD* ⇒ biofilm formation positive regulator

CysB - ExoS SpsC ⇒ secretion/production negative regulator

CysB - pC *exs* C B A ⇒ ExsA ⇒ pC activity negative regulator

58

Régulation par les produits sécrétés

A High Ca²⁺, secretion channel closed B Low Ca²⁺, secretion channel open

Transcription repressed Transcription depressed

⊖ = ExsD ⊗ = ExsC ⊙ = ExsA X = hypothetical secreted protein

Dasgupta et al. Mol Mic 2004,53: 297

59

La prolifération

Croissance en bio-film ou mucoïdie

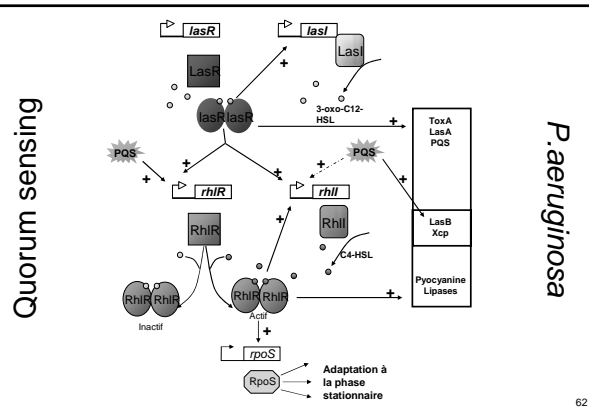
60

Les phases de l'infestation



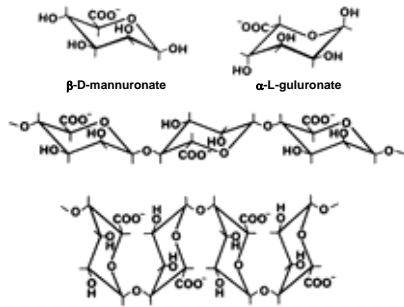
Hassett et al. Adv Drug Deliv Rev 2002,54:1425

61



62

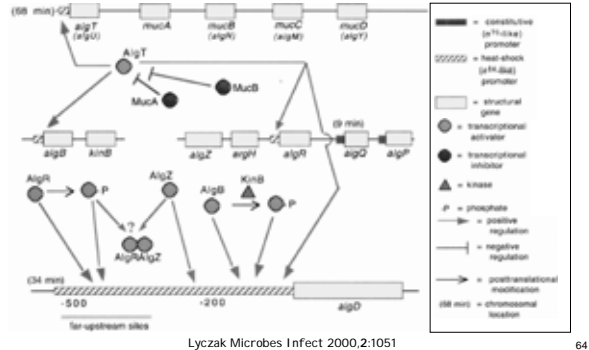
Mucoïdie: synthèse d'alginate



Gaceta Microbiologia 1998,144:1133

63

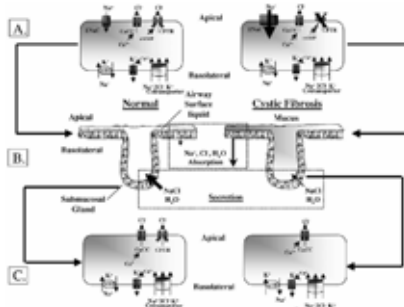
Contrôle de la mucoïdie



Lyczak Microbes Infect 2000,2:1051

64

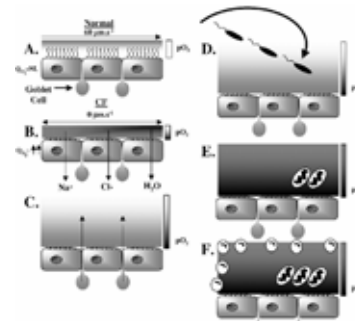
Poumon mucoviscidose



Hassett et al. Adv Drug Deliv Rev 2002,54:1425

65

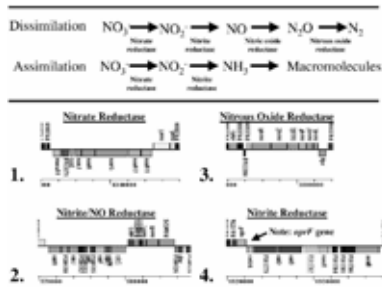
Le bio-film: croissance en hypoxie



Hassett et al. Adv Drug Deliv Rev 2002,54:1425

66

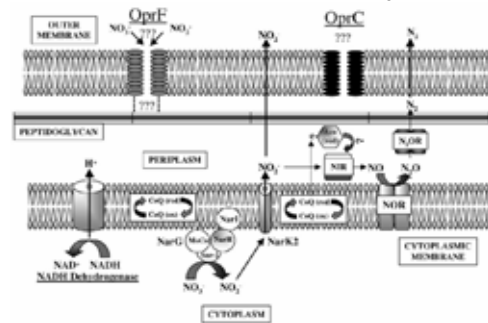
Métabolisme anaérobie



Hassett et al. Adv Drug Deliv Rev 2002;54:1425

67

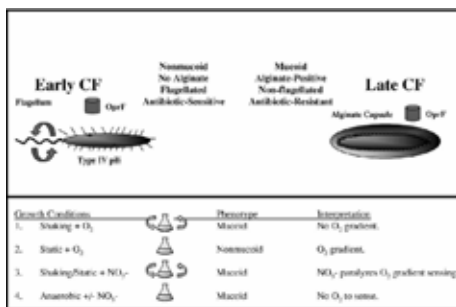
Métabolisme anaérobie



Hassett et al. Adv Drug Deliv Rev 2002;54:1425

68

Invasive ↔ Mucoïde



Hassett et al. Adv Drug Deliv Rev 2002;54:1425

69

Pathogénicité de *P.aeruginosa*

- Phase invasive
 - Adhérence
 - Cytotoxicité (TTSS)
 - Planctonique
- Phase mucoïde
 - Anaérobiose
 - Non cytotoxique
 - Bio-film
- Infection aiguë
- Infection chronique
- Chimio-sensible
- Chimio-résistante

70

Autres modèles pathogènes

Versatilité du TTSS

71

Salmonella spp.

Comment vivre dans un phagocyte

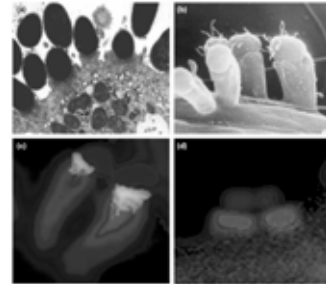
72

Escherichia coli entéropathogène

La stratégie de l'huître

85

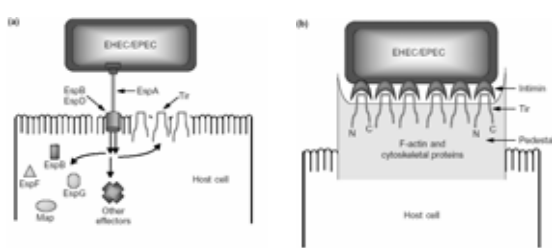
Le piédestal: l'actine détournée



Campellone & Leong Curr Opin Microbiol 2003,6:82

86

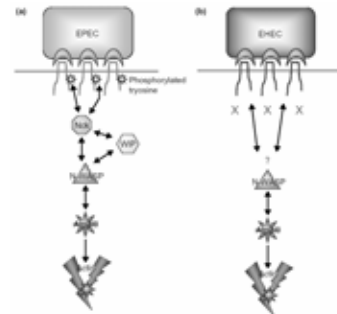
Insertion du récepteur Tir



Campellone & Leong Curr Opin Microbiol 2003,6:82

87

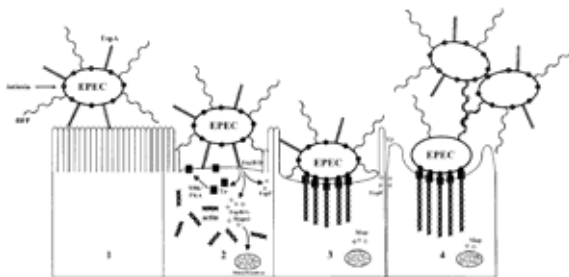
Activation de l'actine



Campellone & Leong Curr Opin Microbiol 2003,6:82

88

Les 4 étapes de la pathogénie EPEC



Clarke et al. Clin Microbiol Rev 2003,16:365

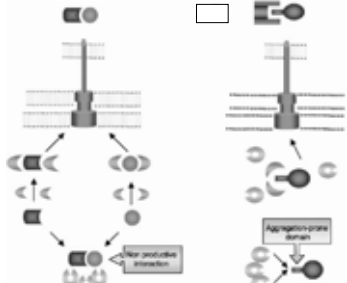
89

Hiérarchisation des toxines

Rôle des chaperonnes

90

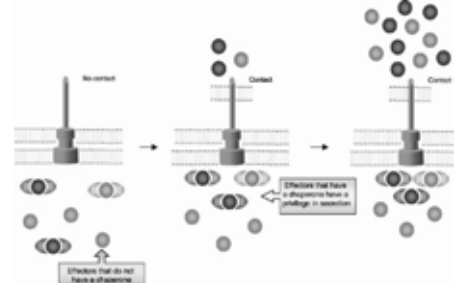
Les chaperonnes du SSTT: facteurs anti-agrégants et stabilisants



Feldman & Cornélius FEMS Microbiol Lett 2003,219:151

91

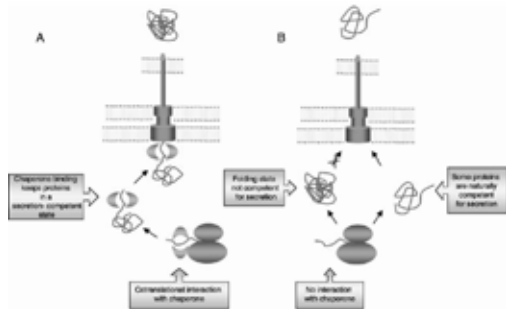
Les chaperonnes du SSTT: signal de hiérarchisation de sécrétion



Feldman & Cornélius FEMS Microbiol Lett 2003,219:151

92

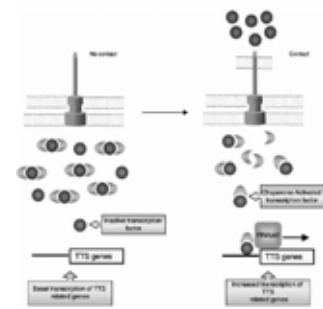
Les chaperonnes du SSTT: maintien des effecteurs non repliés



Feldman & Cornélius FEMS Microbiol Lett 2003,219:151

93

Les chaperonnes du SSTT: régulateurs de l'expression du SSTT



Feldman & Cornélius FEMS Microbiol Lett 2003,219:151

94

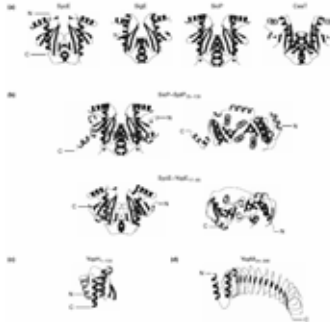
Les chaperonnes du SSTT: organisation génique



Page & Parsot Mol Microbiol 2002,46:1

95

Structure des chaperonnes et effecteurs



Parsot et al. Cur Opin Microbiol 2003,6:7

96

Chaperone alignment

```

SicP (1) M1SHOETA2NESEK3Q4PLTF5DNNO6CLL7---8SD9FTT10EA11N12D13Q14LE15N16
SicE (1) --17ME18LD19NE20VD21ALG22LD23AD24ED25--26RP27LE28LD29---30D31GV32VE33NE34S35D36HT37EX38CC39
CesT (1) N40SS41EL42LD43EP44AK45TG46IG47IS48FN49ML50CS51PI52DE53IT54YI55CS56DA57N58DE59N60MY61
SycE (1) N62SP63KA64EP65PO66Q67SG68IS69TS70VE71Y72---73G74PA75CE76ET77RE78Y79---80GL81ME82
OfeI (1) MN83PL84Y85RA86TH87DF88LA89LD90PT91ND92ES93VE94EC95Q96---97G98PE99L100CH101AA102PT103D104RL105ME106

SicP (51) M1EL2PL3SP4CG5DS6IM7RO8EW9VING10EL11ANN12---13RC14L15AY16DA17RE18TL19LI20HAI21---22DD23T24
SicE (45) P25PE26LP27---28DD29IL30TG31Q32IR33NY34TC35---36AP37ES38AD39AT40AK41AL42Y43EL44---45DT46
CesT (54) M47CC48FP49TD50---51N52NP53FA54LE55EL56NAN57LF58ENG59---60GY61LC62Y63SG64RC65LL66AL67RF68---69LD70DA71
SycE (51) EP72S73---74LD75NN76EE77---78ET79ES80SH81IF82---83Q84DL85K86IL87---88WE89VG90CP91W92ML93NR94CP95---96NN97ED98
OfeI (52) RL99SS100---101CG102AT103AS104SN105EP106---107SP108CP109CP110---111TL112EP113---114ES115SN116RE117---118GL119LD120

*
SicP (102) NT1YH2EL3ES4ES5FN6OC7EA8LN9IL10Q11---12EY13AK14Y15
SicE (90) TE16E17AL18Q19FE20EL21ES22SV23OL24RE25RY26---27
CesT (105) TP28RE29EL30---31EL32W33VA34SN35ML36LV37SN38Q39IL40FN41SN42KI43E44ES45SS46DN47K48HT49TA50GR
SycE (100) IN51NS52HY53COL54ML55VO56GA57RL58CT59SL60IS61PP62RS63FS64---65
OfeI (98) RA66Q67HH68DE69RC70W71AA72AE73LE74---75

```



Chaperone form dimers
Interaction with the N-terminal domain of type III toxins

Strebins & Galan, Nature 2003

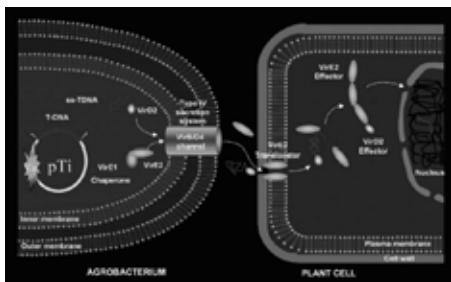
97

I take my chaperon to the play –
 She thinks she's taking me.

From *The chaperon*
 by Henry Cuyler Bunner
 (1855 – 1896)

98

Carcinogénèse bactério-induite: *Agrobacterium tumefaciens*



Duckely & Hohn FEMS Microbiol Lett 2003,223:1

99

Conclusion

- Chaque bactérie a développé sa propre stratégie en fonction de ses hôtes
- La pathogénicité est en grande partie liée à l'hôte lui même
- Les mêmes stratégies sont développées
 - Que l'hôte soit animal ou
 - Que l'hôte soit végétal

100

GREPI

Prof. F. Morel

Biotechnology group

Prof. B. Polack

- Bacterial vectors
 - **B. Polack**, L. Quééné, D. Filopon, O. Épaulard, M. Bonnet, D. Lamotte, B. Toussaint, DK. Shen
 - *P. aeruginosa* optimization
 - BP, LQ, DF, MB, DL, DKS
 - Dendritic cells
 - BT, OÉ
- Protein vectors
 - **B. Toussaint**, JL. Lenormand, B. Marques, L. Crombez
 - Proteo-liposomes
 - JLL, BM
 - Protein chariots
 - BT, LC
- Lentiviral vectors
 - **G. Pernod**, F. Solly, A. Michoud, N. Bolle

101