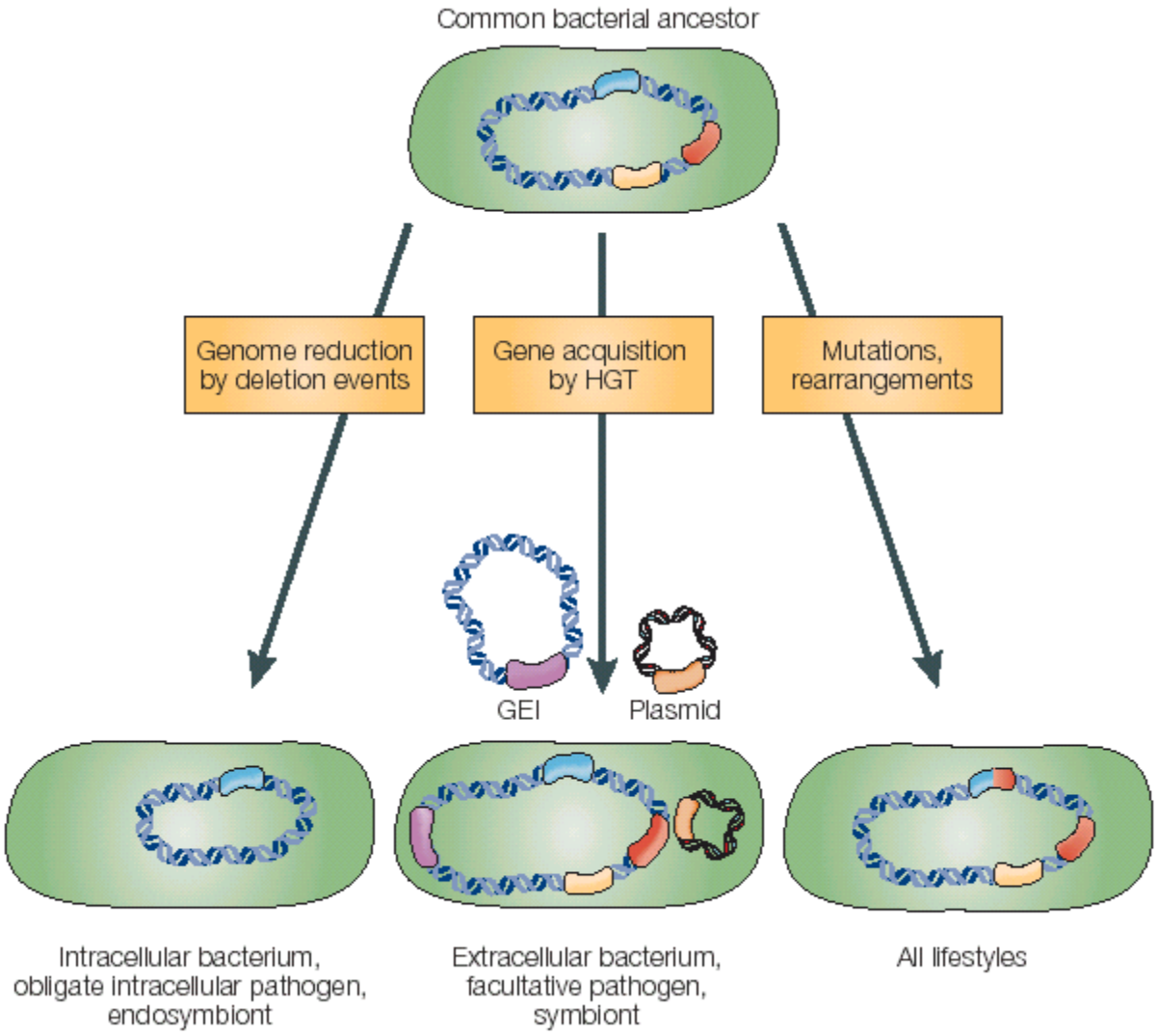
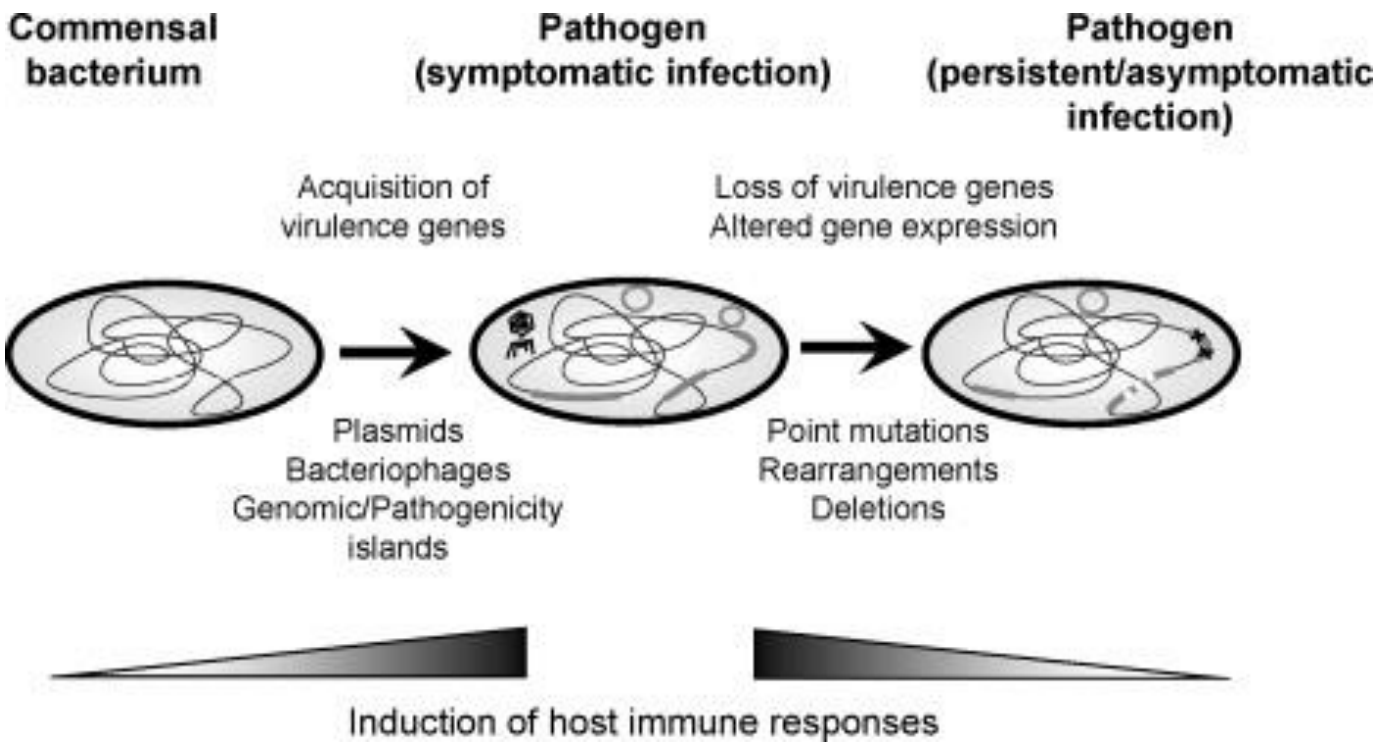
Evolution of a bacterial pathogen: major strategies



Impatto della plasticità genomica nell'adattamento dei batteri patogeni



L'acquisizione di elementi genetici mobili quali batteriofagi, plasmidi o isole genomiche contribuisce all'evoluzione dei patogeni dalle varianti commensali. Durante l'infezione, la fluidità genomica tramite riarrangiamenti, delezioni o mutazioni puntiformi determina l'insorgenza di ceppi patogeni persistenti oppure determian una down-regolazione di alcuni geni . Nei ceppi persistenti si nota un accumulo di mutazioni.

Genoma di *E.coli* : esempio di grande variabilità

La sequenza dell'intero genoma di E.coli ha rivelato una variabilità intraspecie estremamnte elevata

Sono disponibiliti 4 sequenze genomiche di E.coli

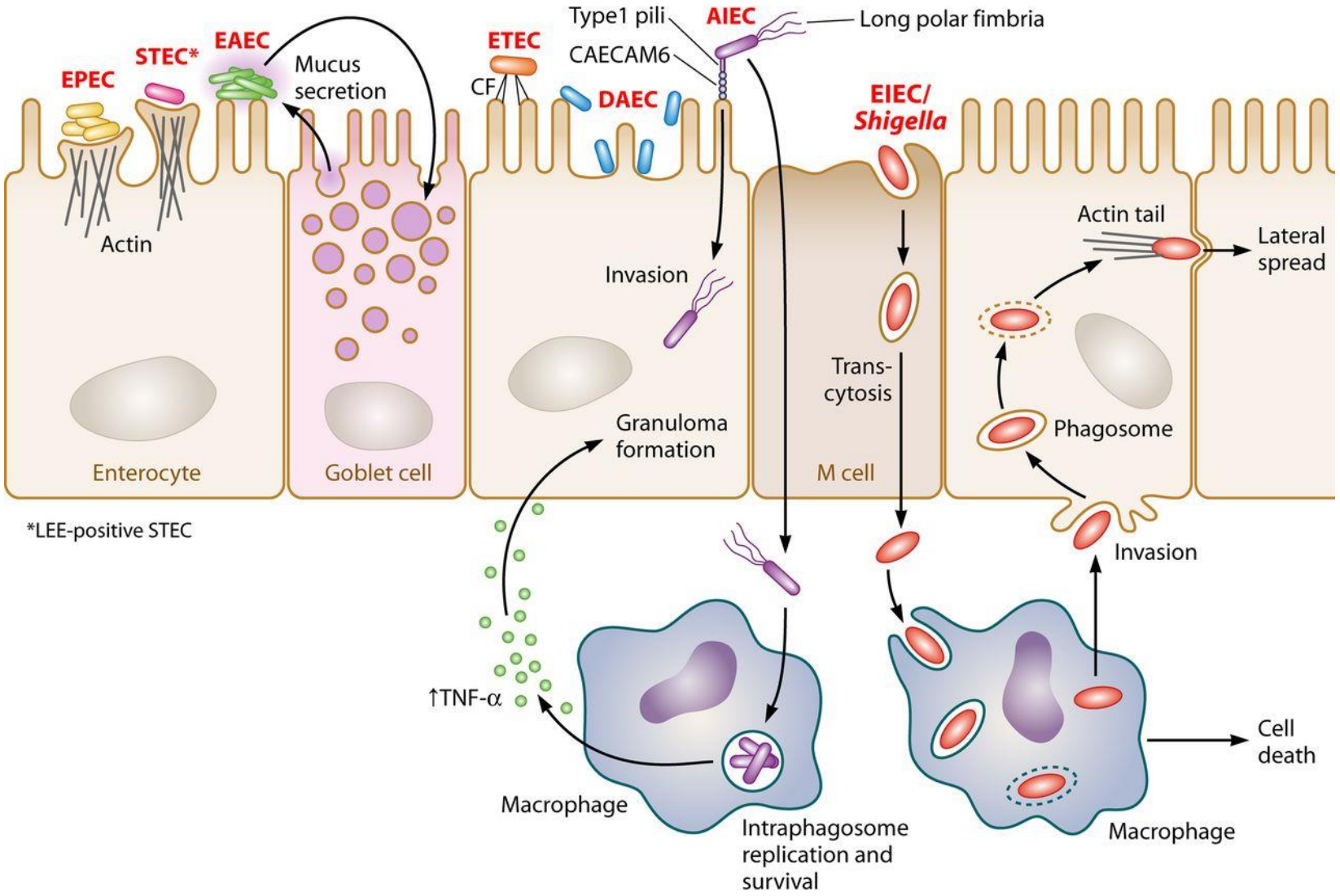
Analisi genomica comparativa ha rivelato che *E.coli 0157* ha un genoma di 1 Mb più grande di quella di *E.coli K12* e circa 25% dei geni non sono conservati nel genoma di *E.coli K12*.

Molti dei geni presenti in 0157 si pensa siano stati acquisiti tramite eventi di trasferimento orizzontale e tramite elementi genetici mobili quali fagi, profagi e sequenze IS

Soltanto 3.000 geni sono in comune tra i 4 genomi di E.coli mentre erano 4.000 tra E.coli K12 e 0157

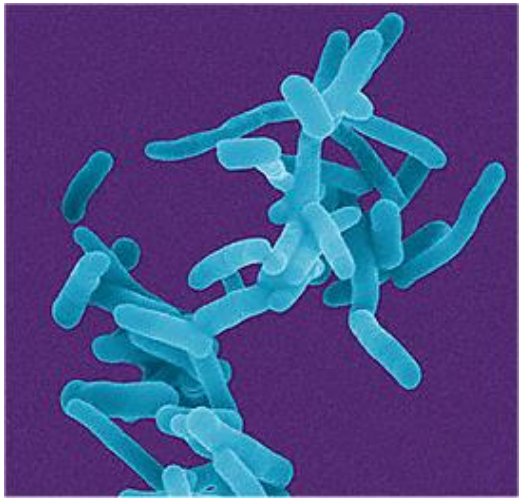
I 3000 geni comuni presentano SINTENIA suggerendo una base di trasmissione verticale

Adherence patterns of enteric E. coli.



**Matthew A. Croxen et al. Clin. Microbiol. Rev. 2013;
doi:10.1128/CMR.00022-13**

Shigella



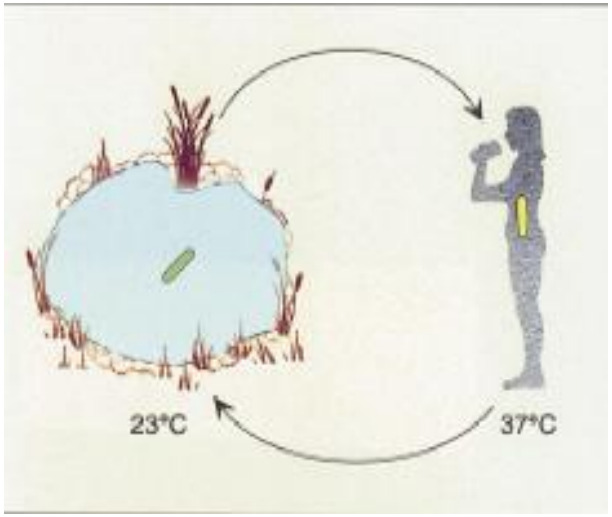
- is a Gram negative, facultative anaerobe
- is an intracellular pathogen
- is the etiological agent of bacillary dysentery, an acute diarrheal disease
- causes 160 million of episodes, determining 1.1 million deaths/year in children and infants in developing countries.

Infection is spread via fecal-oral route

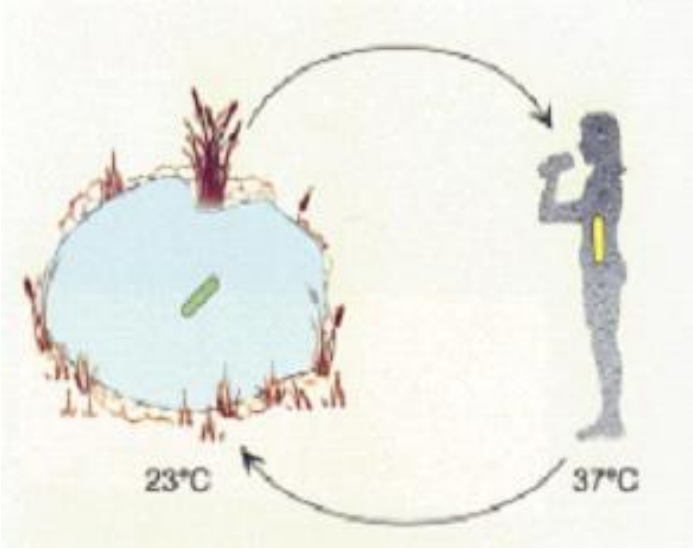
Subgrouped into four "species":

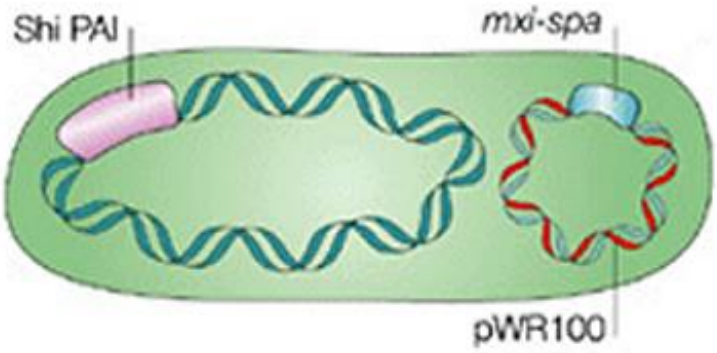
- *Shigella flexneri*
- *Shigella dysenteriae*
- *Shigella boydii*
- *Shigella sonnei*

Due to the high level of genome homology, *Shigella* is now considered among *E.coli*



Shigella





- is an intracellular pathogen causing human dissentery, a highly infectious disease
- is able to invade epithelial host cells and to manipulate the immune cell functions
- is able to survive in the outer environment and is acquired mainly from contaminated water
- shares a high genome homology with *E.coli* (belongs to the same pathovar)
- has a genome characterized by a large pINV plasmid, acquired by HGT during the transition towards pathogenicity

Shigella è ormai considerato uno dei modelli di *E. coli* patogeni

Organizzazione strutturale del genoma di Shigella

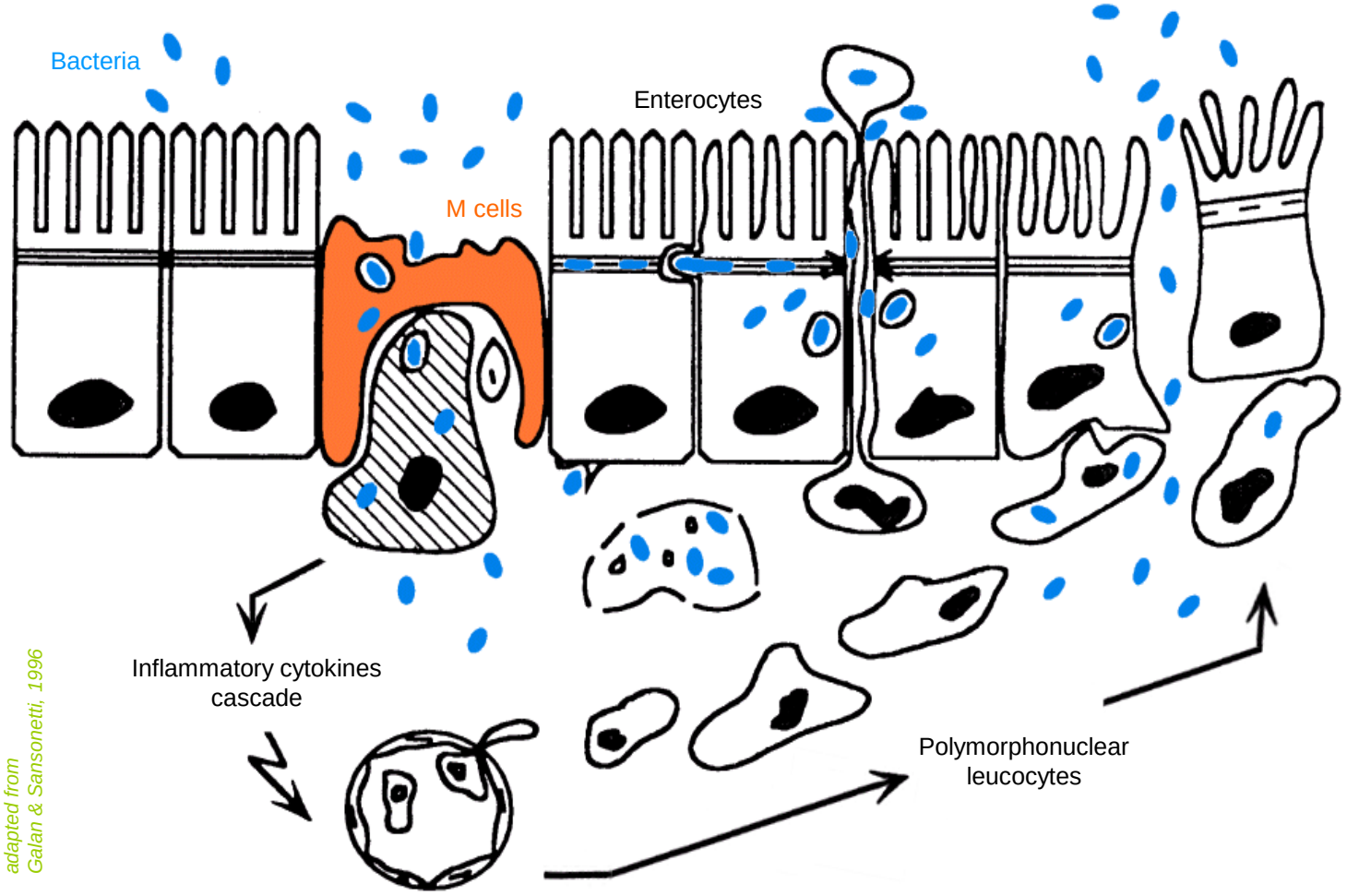
Un singolo cromosoma circolare di 4.599.354 basi
leggeremente più piccolo rispetto ad E.coli K12 4.639.221

L'organizzazione riflette quella descritta per *E.coli* 0157 e per gli *E.coli* UTI :
larghe regioni di colinearità interrotte da isole genomiche acquisite per HGT (15 riarrangiamenti sup. alle 5 Kb)

7 operoni rRNA organizzazione alterata rispetto a E.coli K12
98 geni tRNA tra i quali 3 copie di un nuovo cluster di 4 tRNA ciascuna inserita in un profago .

Ampie inversioni nelle regioni **ORI** e **TER**, probabilmente IS mediate

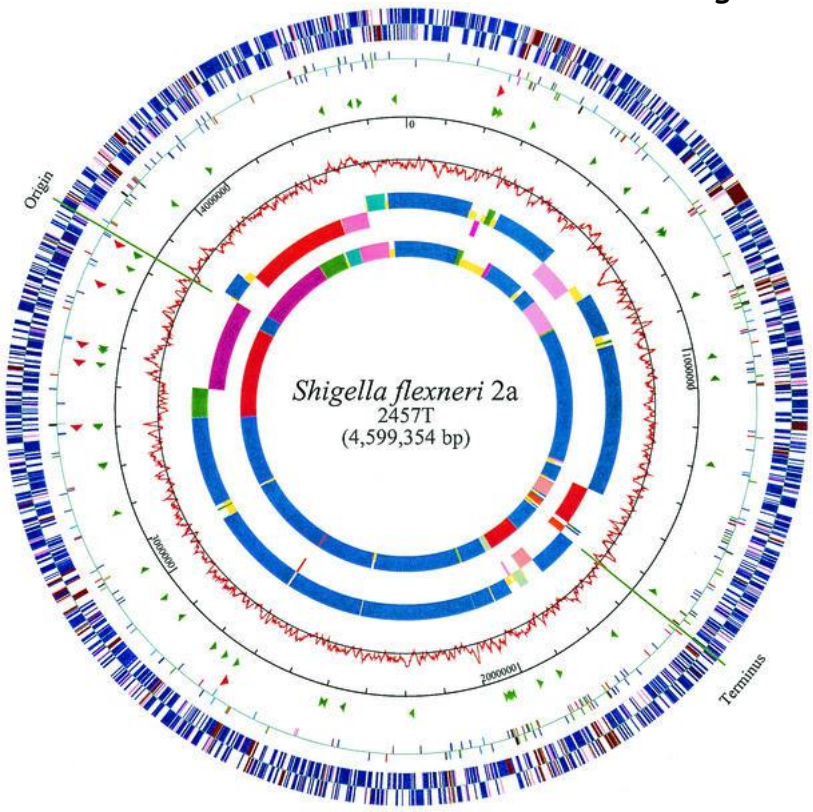
Model for *Shigella* invasion of the colonic mucosa



Shigella and *E.coli* genomes are highly homologous

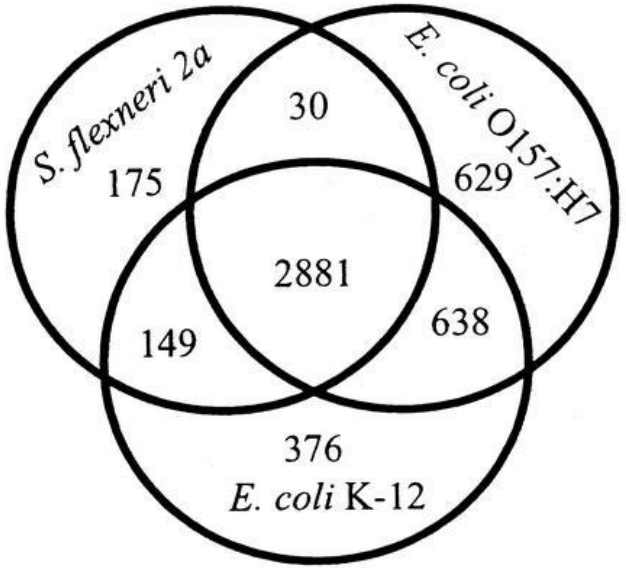
The genome of Shigella

- Large regions of colinearity with the *E. coli* genome
- High percentage of IS (6.7% of the genome, 1.5% in E.coli)
- Extensive gene decay; high fraction of pseudogenes (8%)



genes common to *Shigella* and *E.coli*
genes unique to *Shighella*

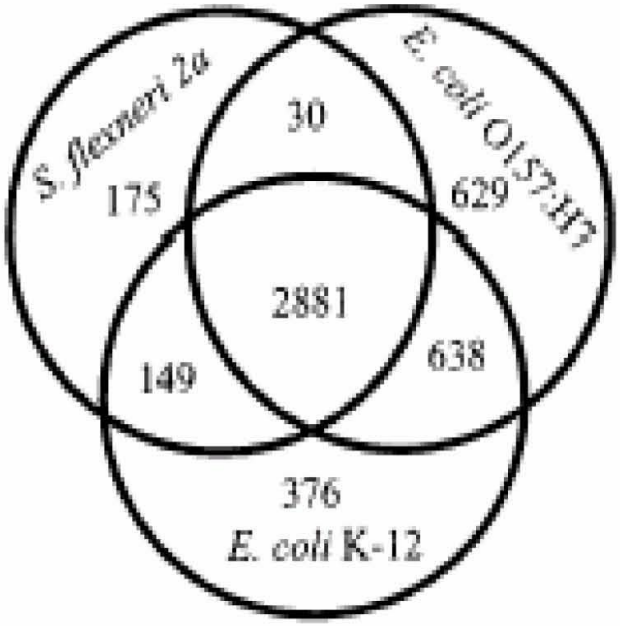
Number of intact proteins

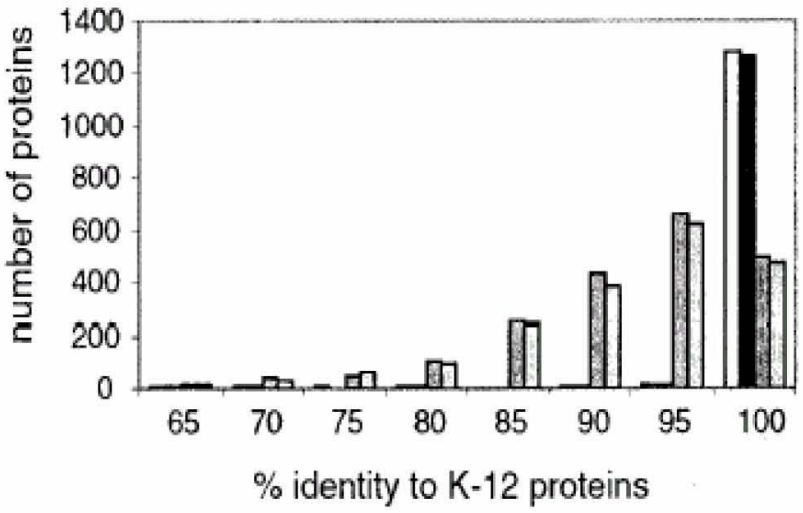


The number of proteins shared by *Shigella* and *E.coli* is very high, confirming the presence of an extensive common backbone

Nonostante le differenze vi è un' elevato livello di omologia tra Shigella e E.coli

Esistenza di un 'ossatura centrale comune



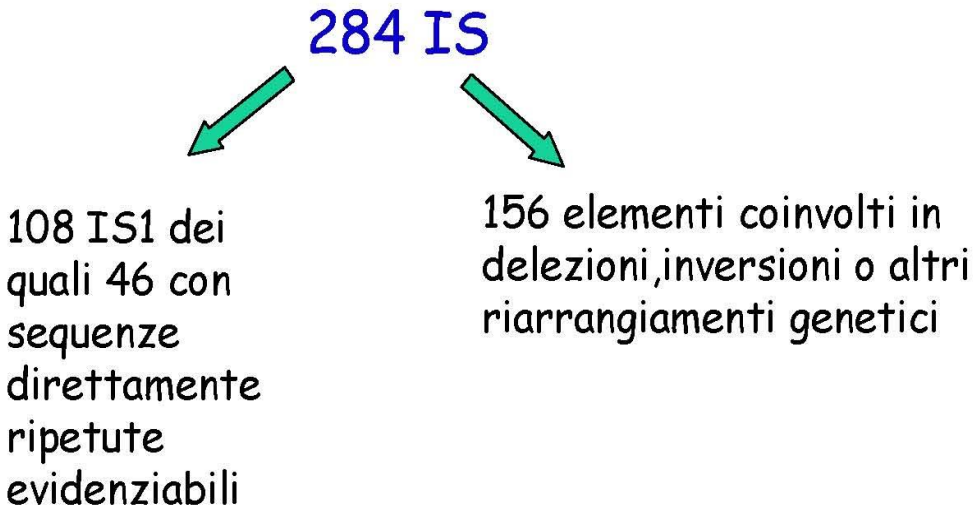


La gran parte delle proteine di *S.flexneri* ed *E.coli* sono identiche vi è diversità invece con le proteine di *Salmonella*

Distribuzione degli elementi IS

Gli elementi IS identificati costituiscono il 6.7% del cromosoma (309.4 kb) in contrasto con gli altri batteri (0-4%).

In E.coli 1.5% in Y.pestis 3%
Unica eccezione Archea *Sulfolobus solfataricus* 10% di IS (genoma di 2.9 Mb)



Quante isole ci sono?

37 isole che codificano almeno un gene (non correlato ad IS)

Mostrano omologia con proteine presenti in diversi microrganismi patogeni per piante, animali e con stili di vita diversi

Categoria funzionale	N.Orf	Specie con omologia
Virulenza	10	*S.flexneri, Y.pestis*
Adesine	7	*Salmonella, E.coli* path, *Actinomiceti*
Regolazione	5	*E.coli 0157, Salmonella*
Metabolismo energetico	31	*Listeria, Caulobacter, Salmonella*
Cattura del ferro	12	*Salmonella, E.coli* path.
Res. a composti organici/ inorganici	7	*E.coli path,Caulobacter,Agrobacterium*
Trasporto	11	*Salmonella, E.coli path*
Struttura cellulare	9	*Salmonella , E.coli path*
Biosintesi/metaboliti centr	17	*Salmonella, E.coli path, V.cholerae*
Funzione sconosciuta	68	*Salmonella, Pseudomonas, Synorizobium*

Plasmidi

Grande plasmide di virulenza circa 218-220 kb
contiene tutti geni coinvolti nel processo di invasività

2 piccoli plasmidi multicopie

In alcuni ceppi plasmide criptico di 165 kb simile al plasmide R27-like di *Salmonella* e al plasmide pMT1 di *Y.pestis*.

PSEUDOGENI

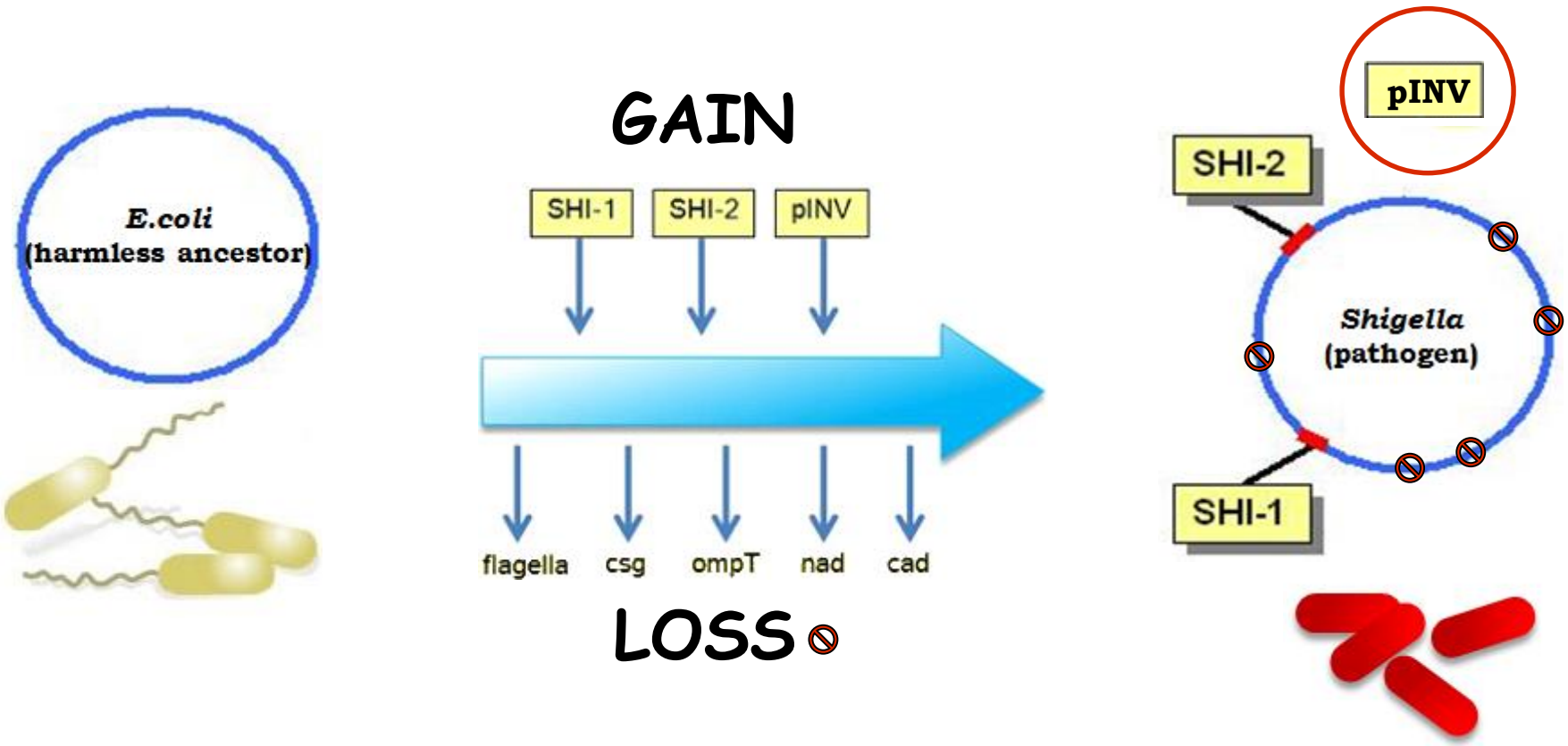
372 geni disrotti (8.1% genoma) ottenuti per :

- perdita di nucleotidi
- mutazioni puntiformi
- delezioni
- riarrangiamenti mediati da IS

879 geni di *E.coli* sono persi:

124	Trasporto
63	Struttura cellulare
62	Regolazione
58	Metabolismo

The evolutionary pathway from *E.coli* to *Shigella*: a good example



The major event that gave rise to the *Shigella /EIEC* pathotype has been the acquisition of the large virulence plasmid (pINV)

Outline

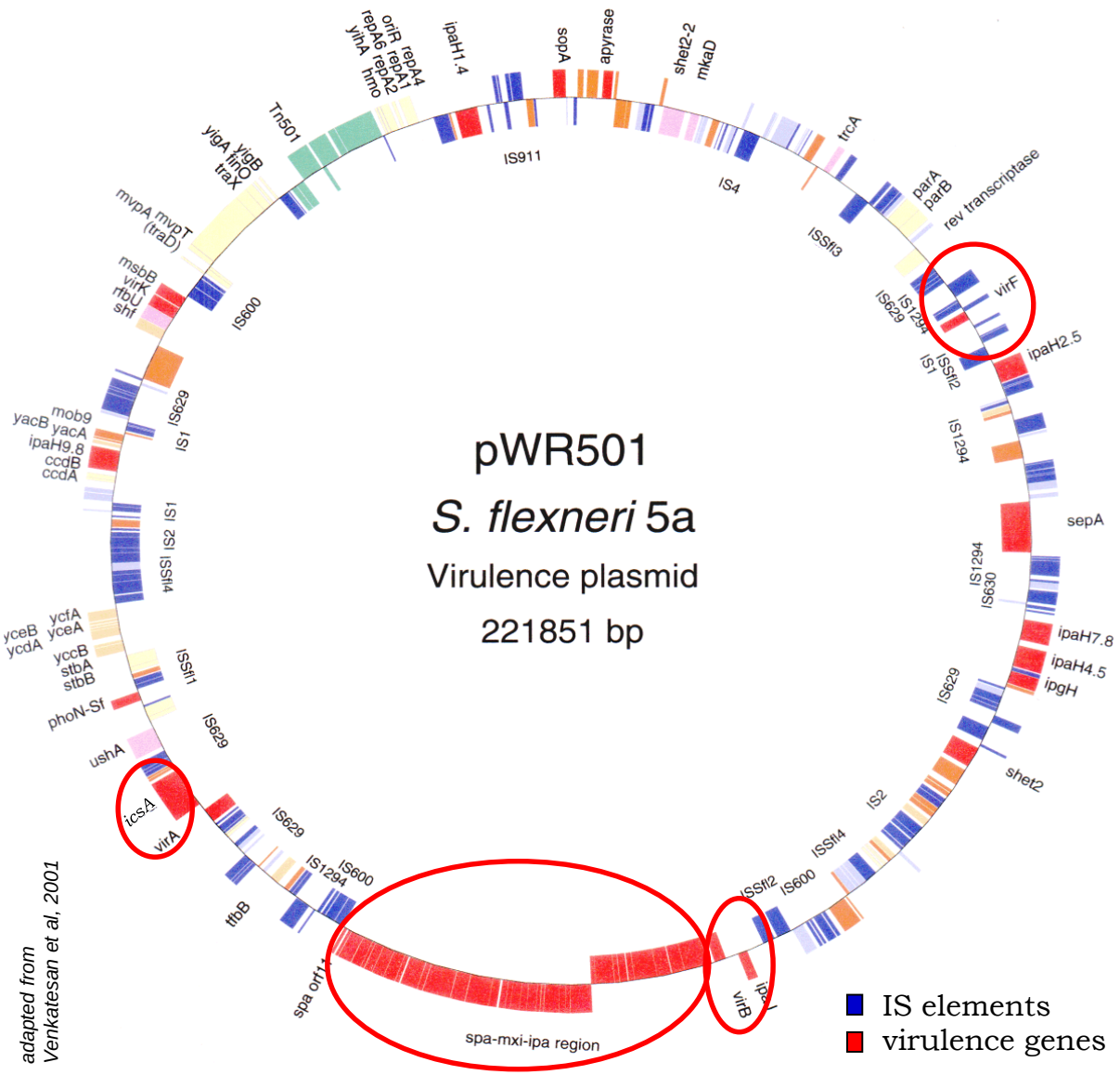
Gain of genes …

- Strategies adopted by *Shigella* to allow the expression of plasmid-encoded virulence genes

Loss of genes …

- Silencing of genes involved in the polyamine pathways
- Loss of genes carried by a lysogenic phage

pINV of *Shigella* contains all virulence determinants



pINV
carries necessary genes for:

- adhesion
- invasion
- spreading

coded by *spa-mxi-ipa* region and *icsA*

- positive regulation

of virulence genes coded by *virF* and *virB*

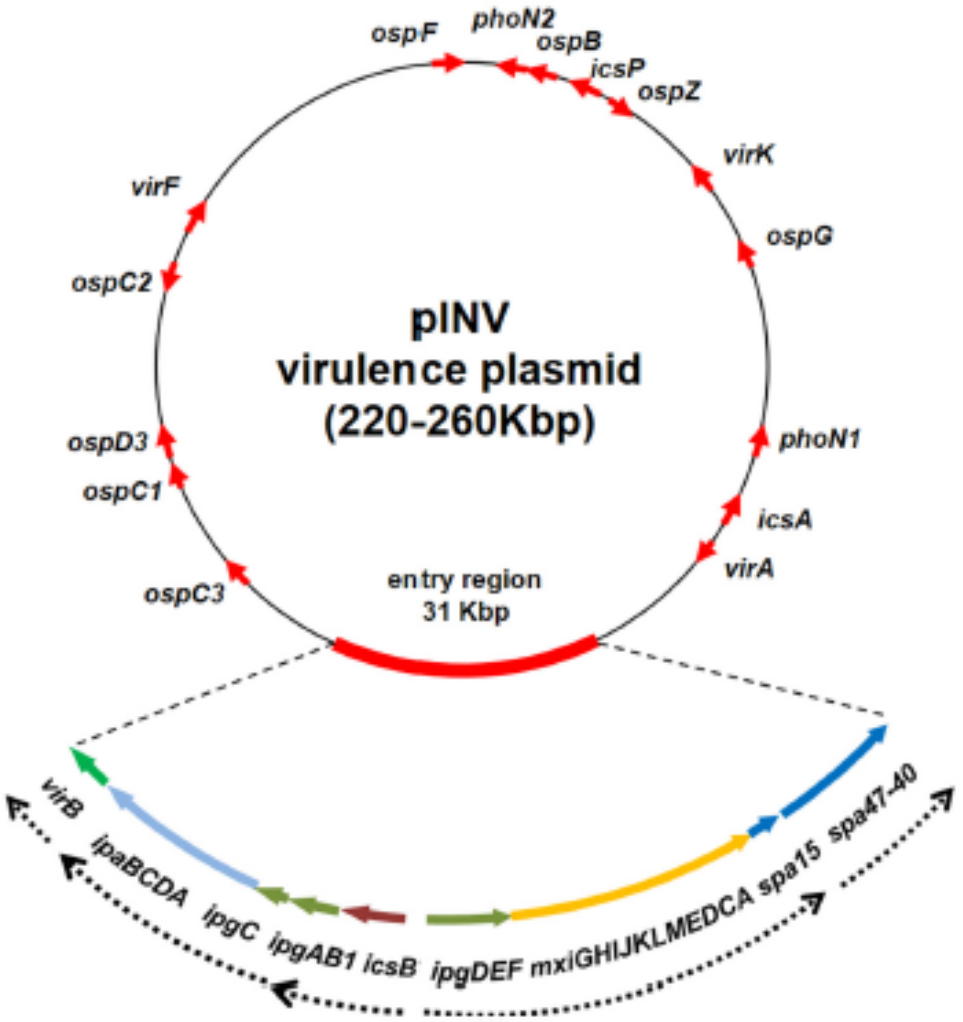
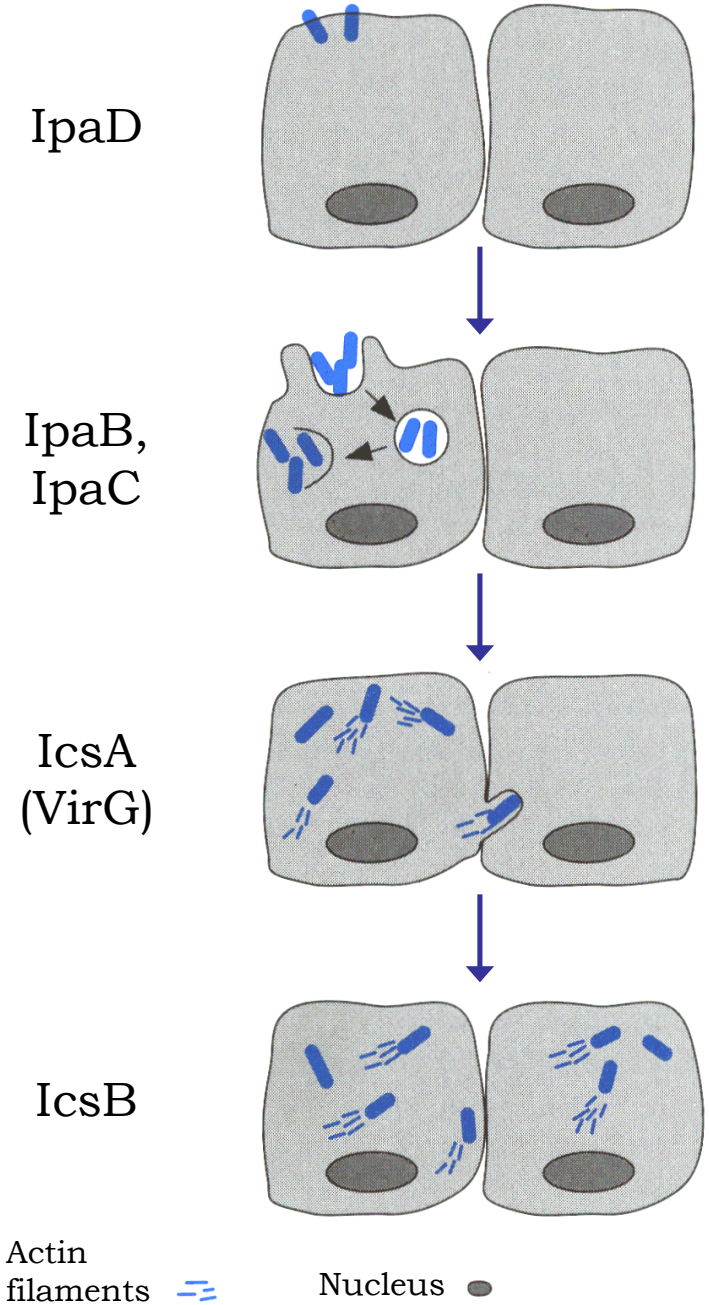
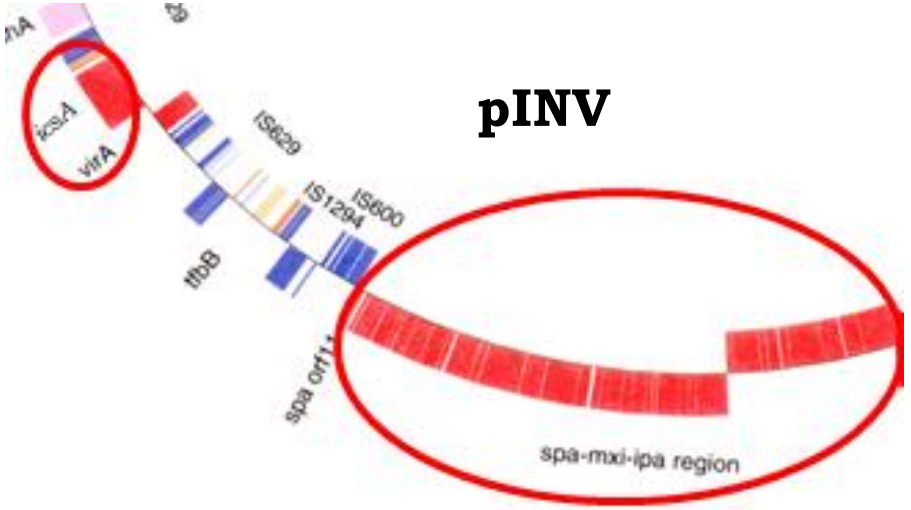


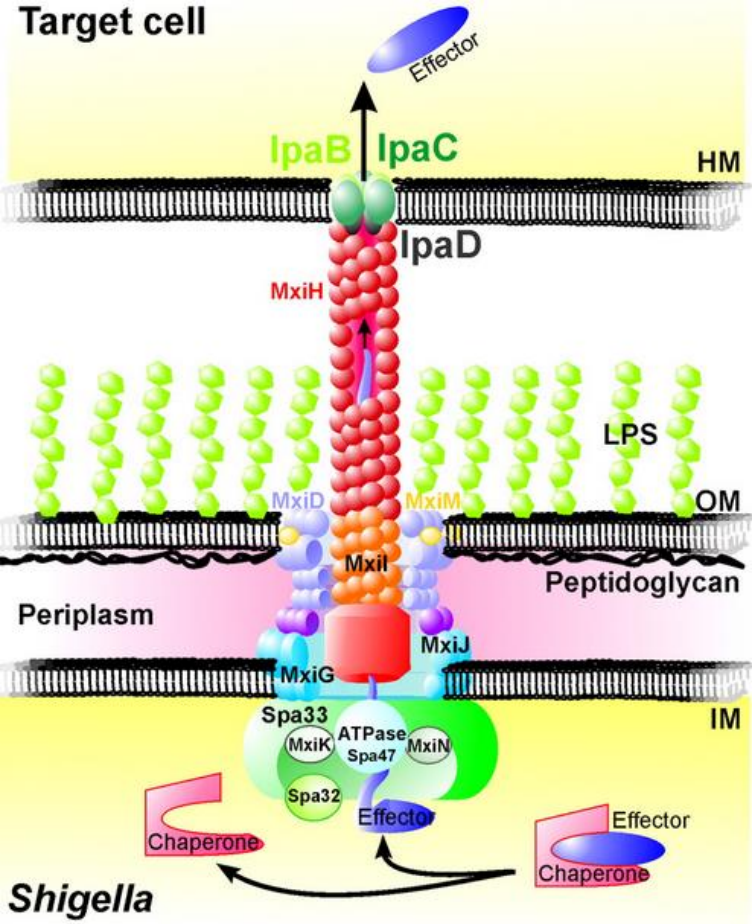
FIGURE 1 | Genetic map of the pINV of *Shigella* and EIEC strains. The red arrows indicate major virulence determinants. Due to the variability in position and number, the *ipaH* genes are not shown. The genetic organization of the entry region is shown in detail, with dashed arrow lines indicating known transcriptional units. The entry region organization is based on the sequence of plasmid pWR100 (Venkatesan et al., 2001) while the entire plasmid is freely drawn to provide the layout of a typical pINV plasmid (the figure is not to scale).



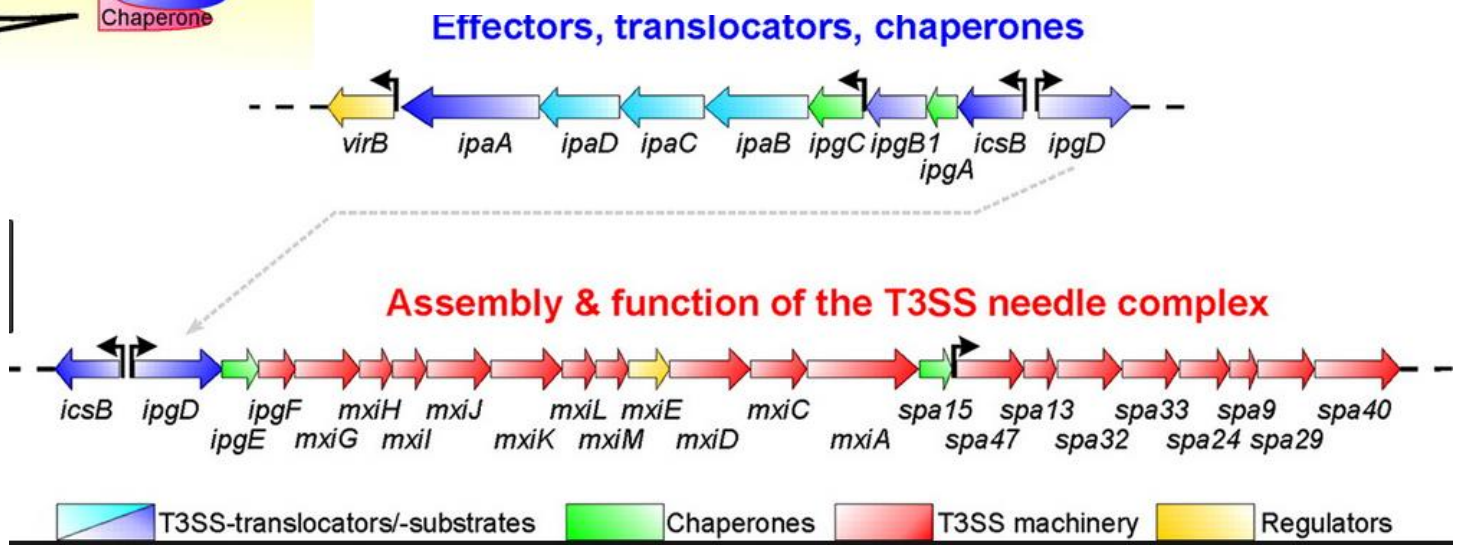
adapted from Salyers & Whitt, 1994



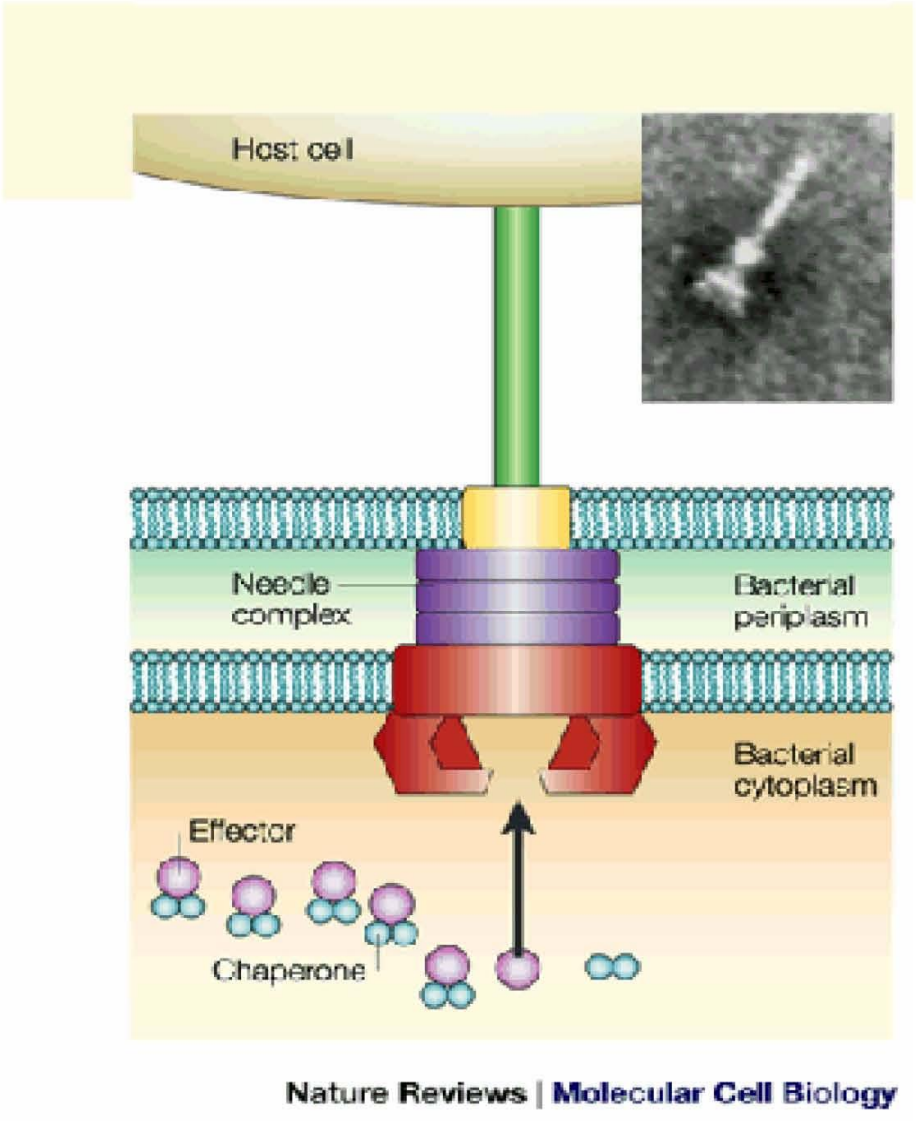
Proteins involved in the invasion process, as well as proteins of Type III Secretion System, are encoded by the virulence plasmid (pINV) and are expressed only at the host temperature (37°C)



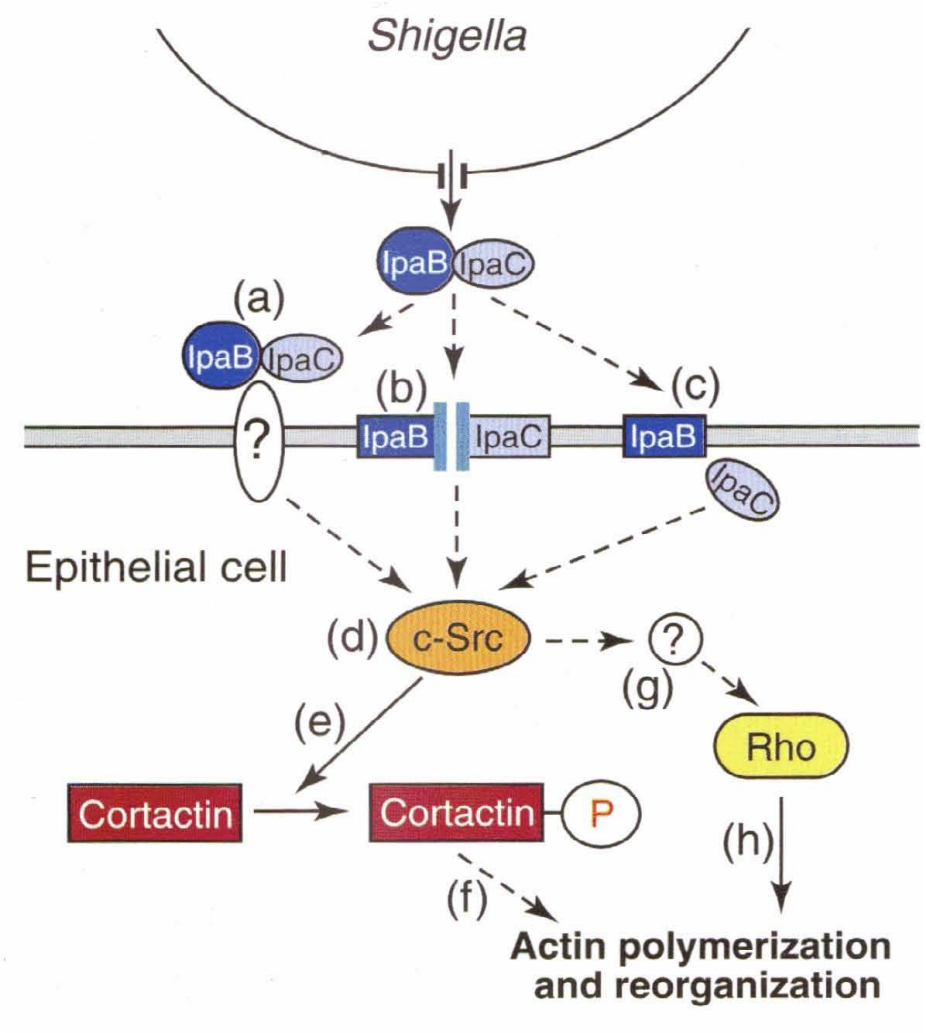
Organizzazione del sistema di T3SS di Shigella



Organizzazione sistema di secrezione di Tipo III: un sistema in grado di iniettare proteine dal batterio direttamente nella cellula bersaglio



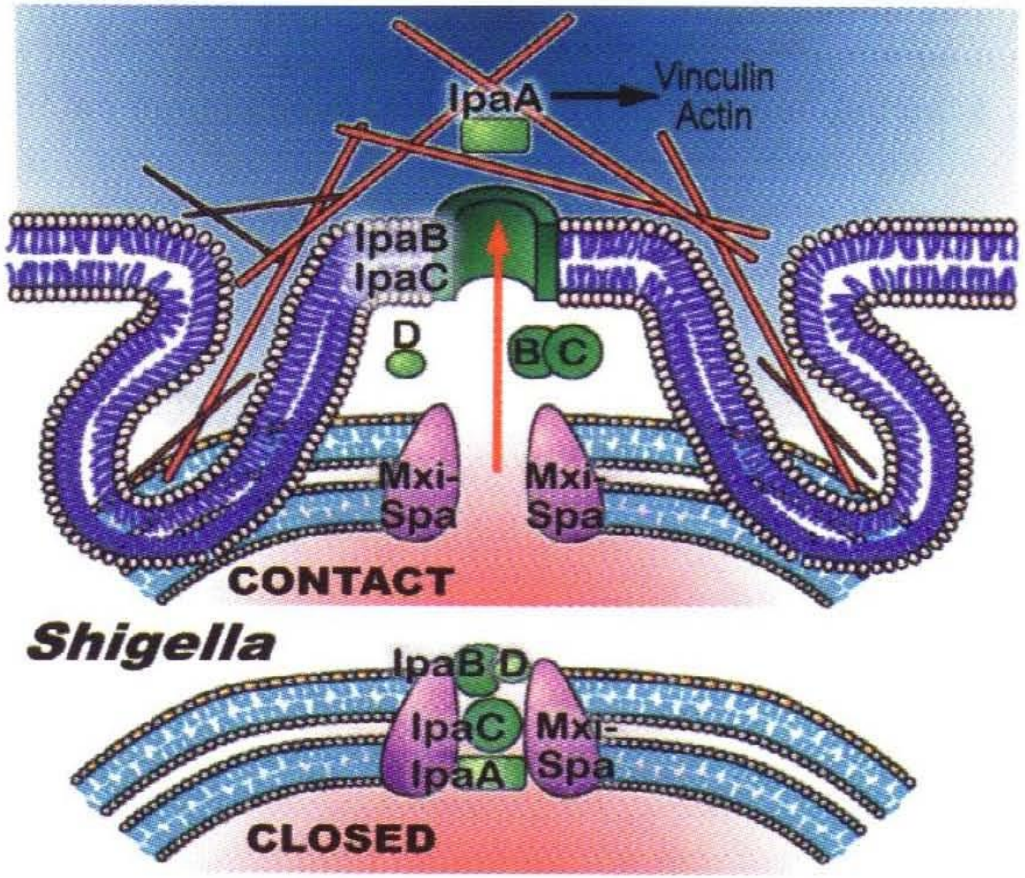
L'iniezione di IpaA nel citoplasma della cellula ospite altera profondamenteil citoschelestro della cellula ospite



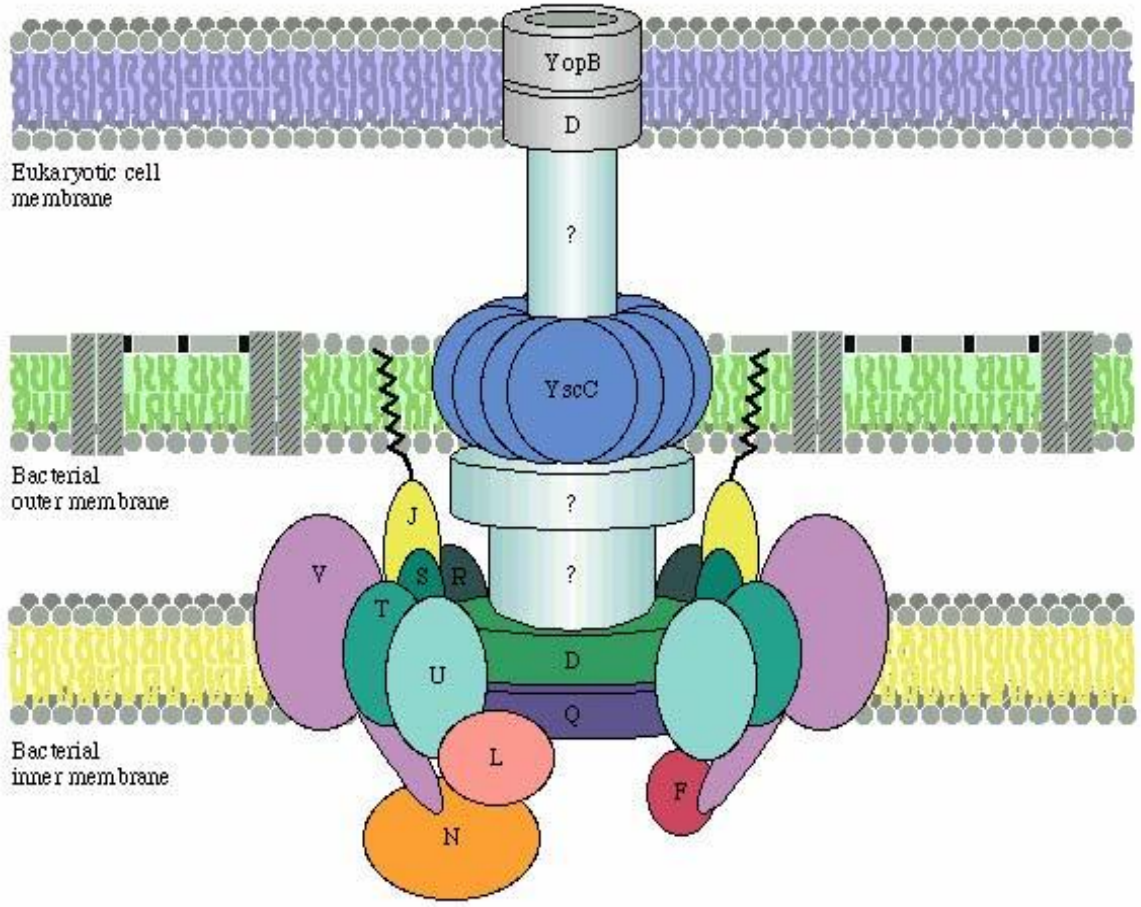
Il sistema di esportazione di tipo III viene attivato dal contatto della cellula batterica con la cellula bersaglio

Le proteine Mxi e Spa formano la struttura transmembranaria del sistema di esportazone

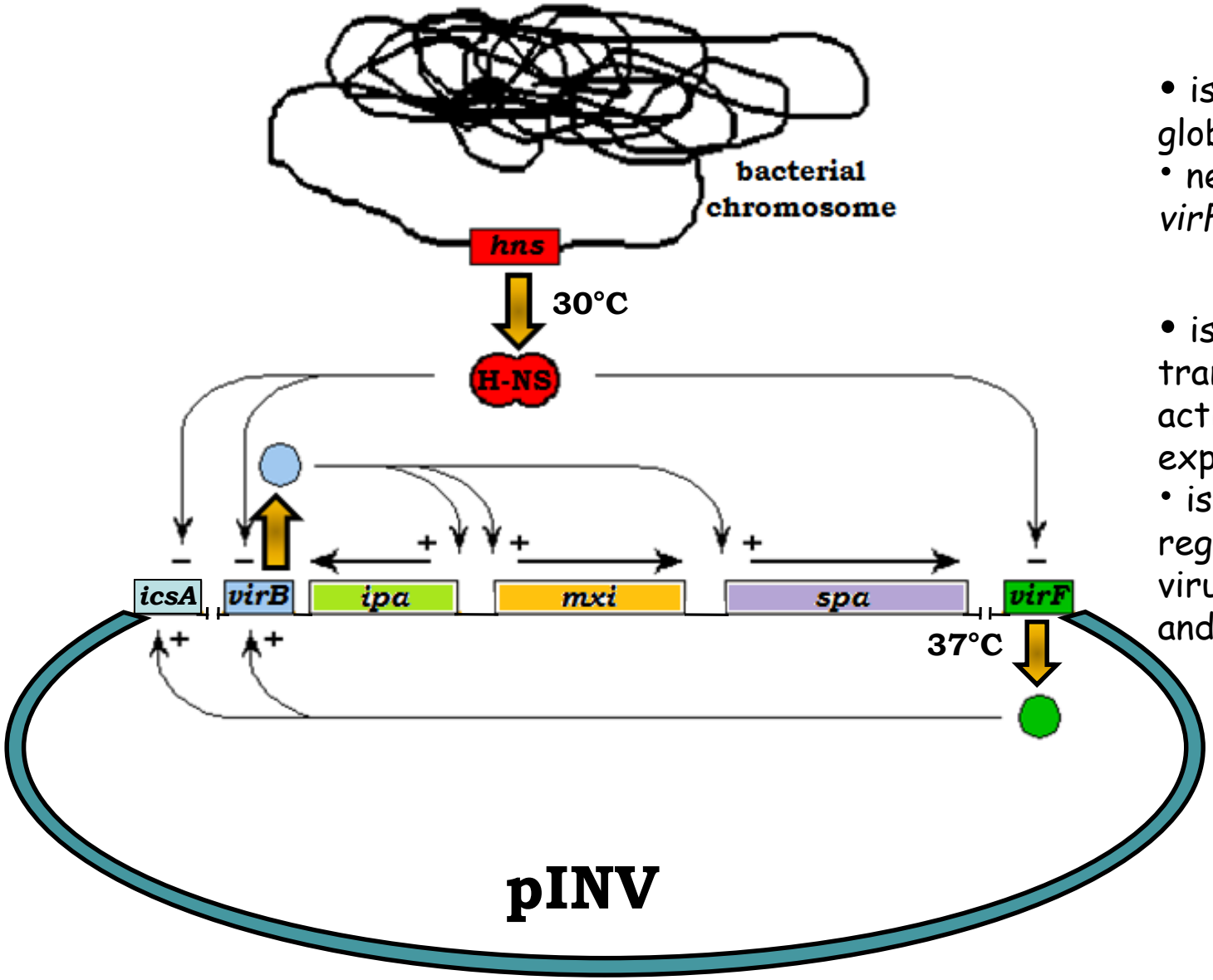
IpaB ed IpaC si inseriscono nella mebrana della cellula bersaglio



Il sistema di esportazione di Tipo III di Yersinia : elevata omologia con il TSS di *Shigella*



Cross-talk between chromosomal and plasmid genes



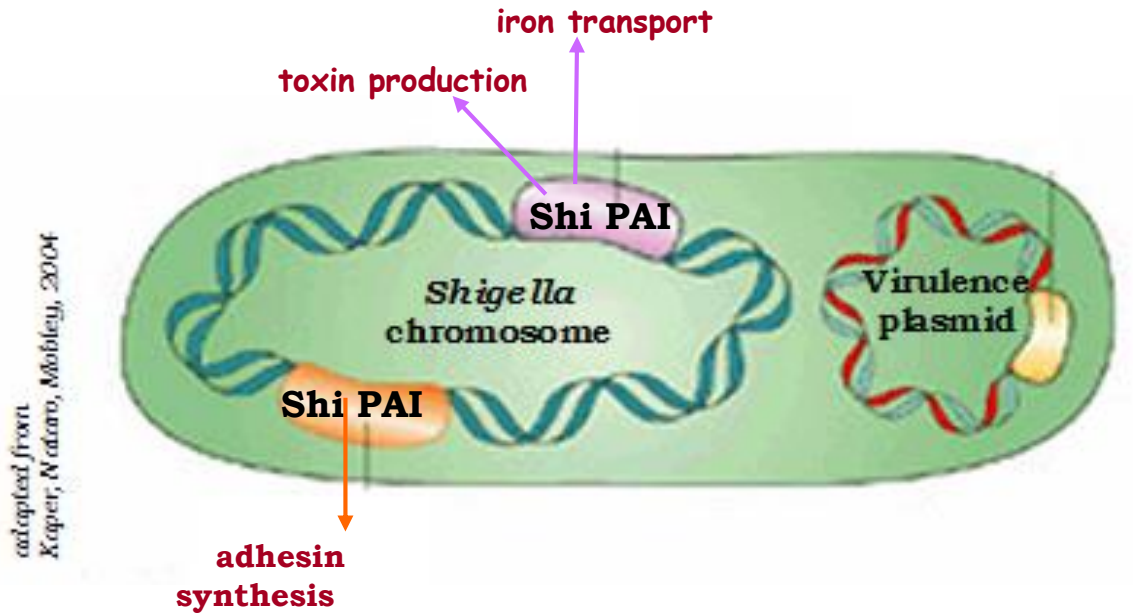
H-NS

- is a chromosomal global regulator
- negatively regulates *virF* and virB at 30°C

VirF

- is the first plasmid transcriptonal activator of *Shigella* expressed at 37°C
- is able to positively regulate plasmid virulence genes, *virB* and *icsA*

Besides the acquisition of the large virulence plasmid pINV, several pathogenicity islands have been identified on the *Shigella* chromosome

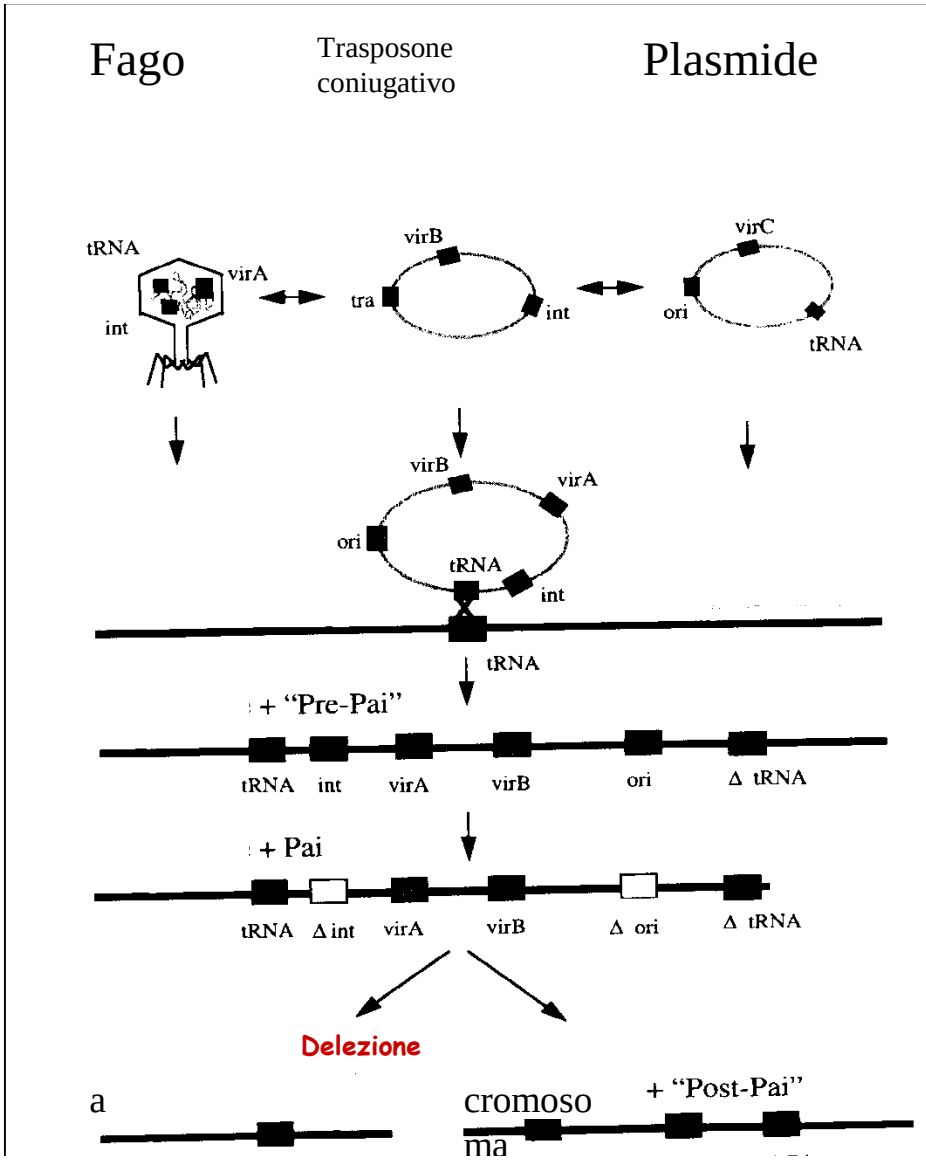


Shi PAIs carry genes that contribute to virulent life style

ISOLE DI PATOGENICITA'

- Contengono uno o più geni di virulenza
- Sono presenti solo nei ceppi patogeni
- Sono di grandi dimensioni 10-200 kb
- Hanno un diverso contenuto in G+C (recente HTG)
- Sono spesso inserite in geni per tRNA
- Sono fiancheggiate da sequenze di DNA direttamente ripetute (DR)
- Sono associate a elementi genetici mobili
- Sono instabili
- Rappresentano strutture a mosaico

Percorso evolutivo nell'origine di una PAI



Formazione di un cointegrato

Trasferimento orizzontale

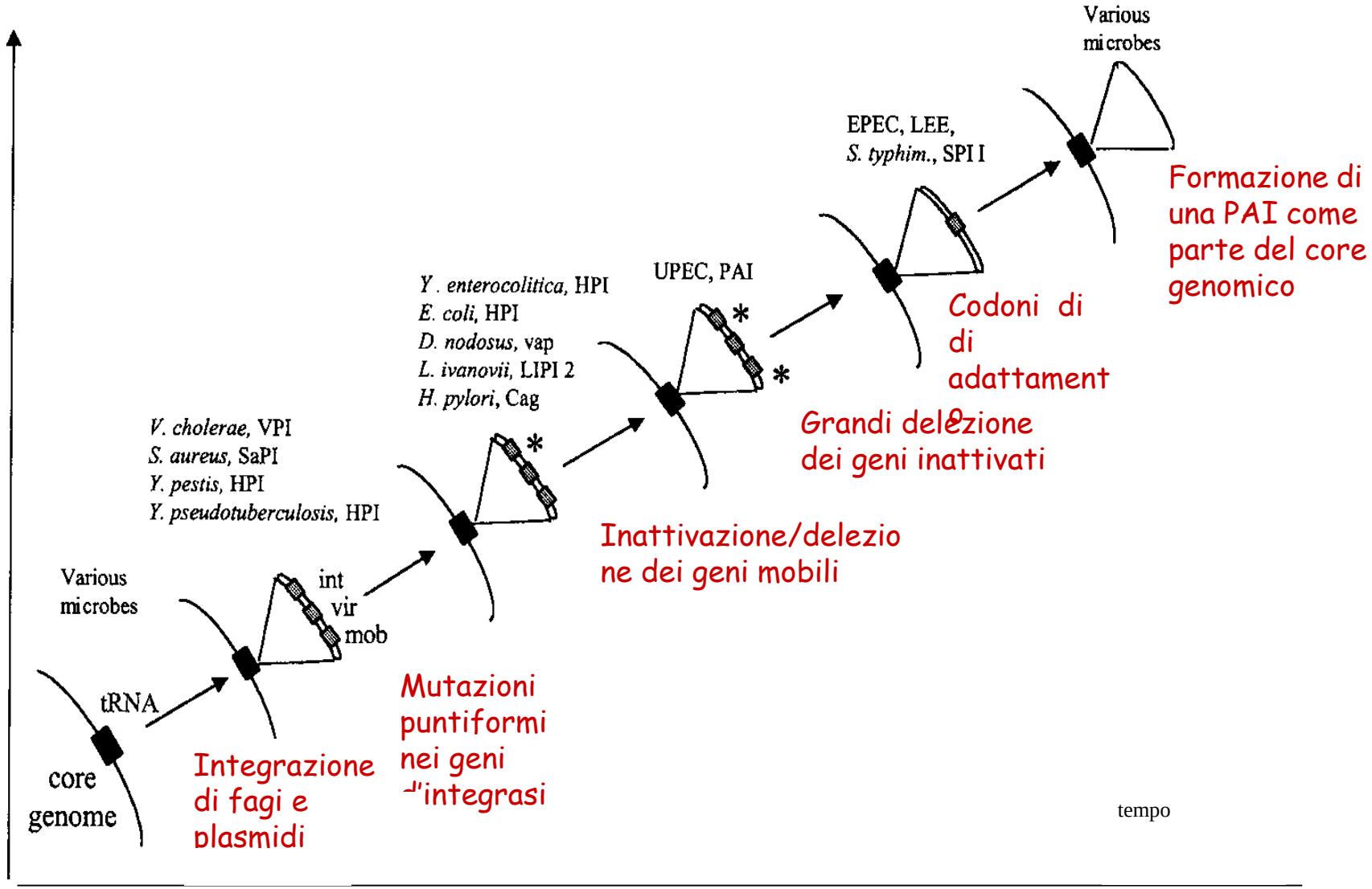
Ricombinazione

Integrazione

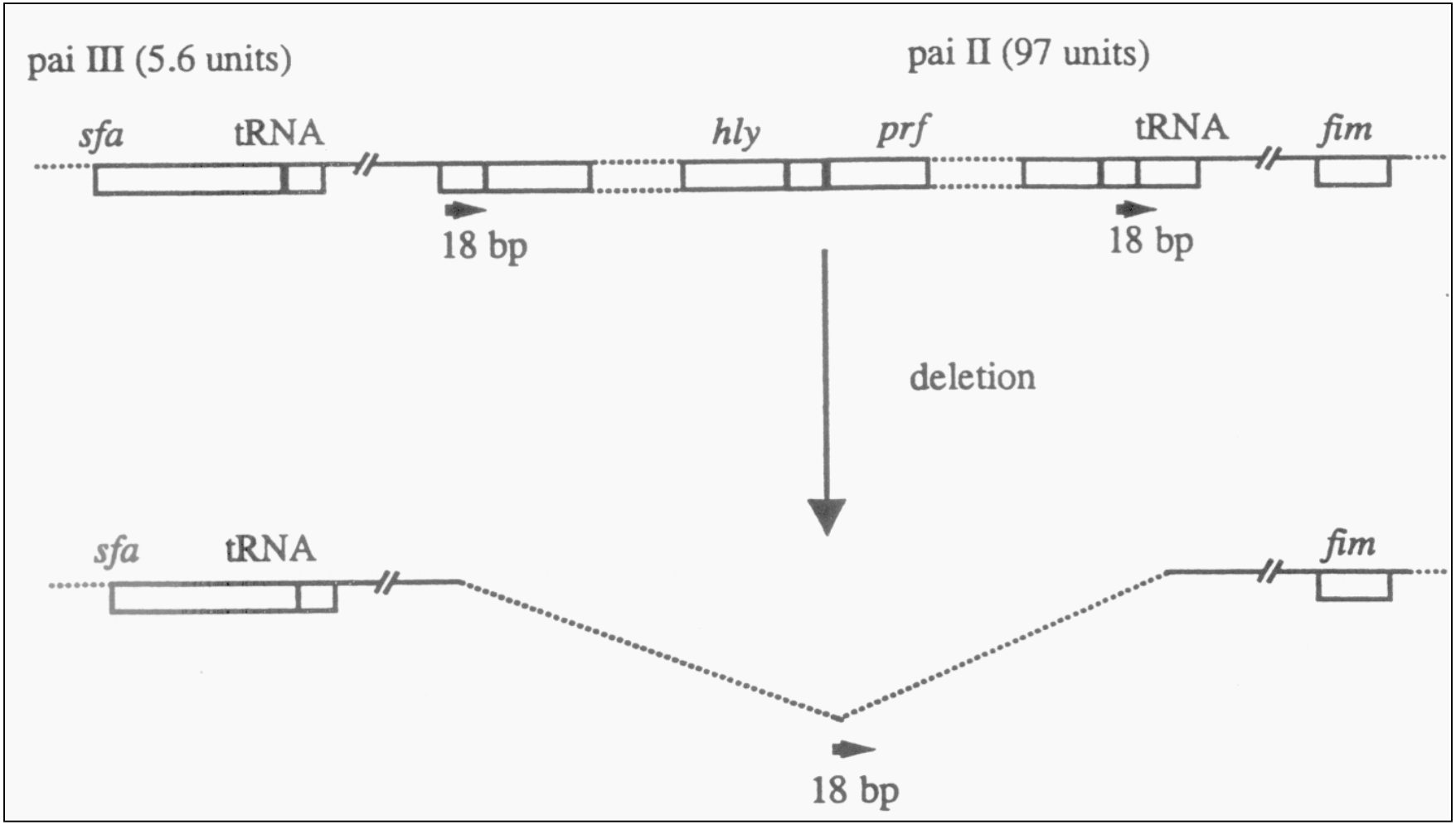
Mutazione, HOMING

Adattamento

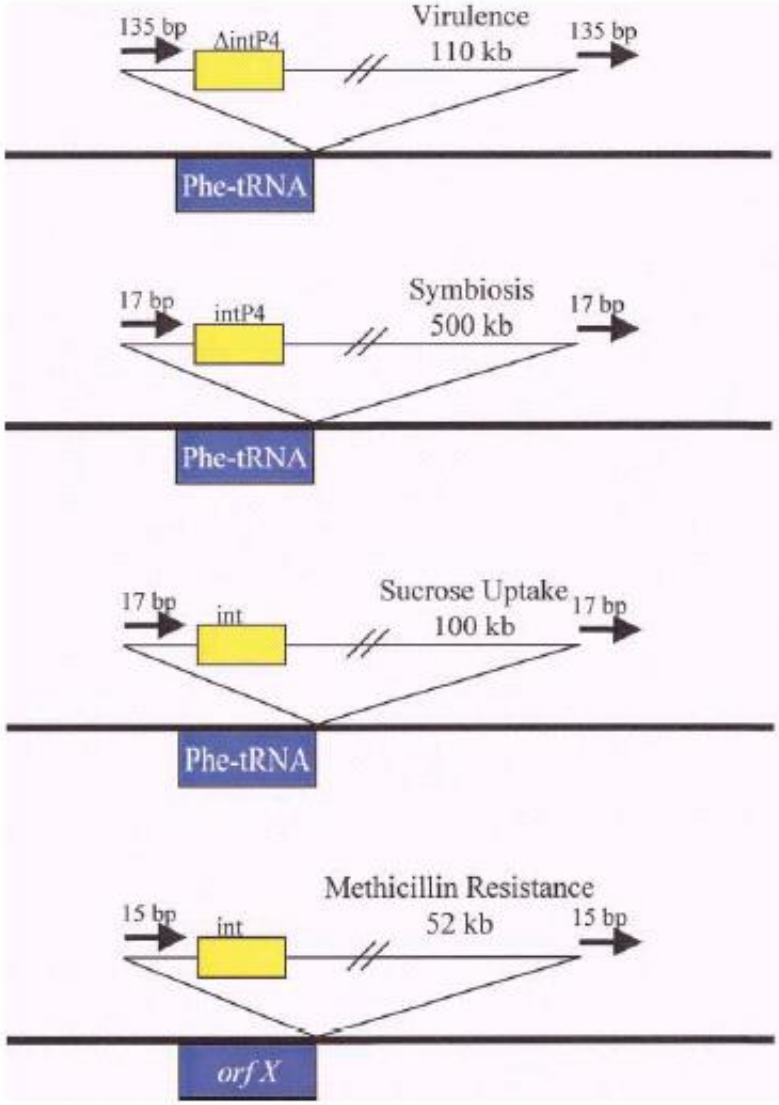
Stadi evolutivi della formazione dell'isola di patogenicità



Le Isole di Patogenicità si possono excidere dal genoma attraverso ricombinazione tra le sequenze presenti nelle sequenze direttamente ripetute (DR)



Non soltanto Pathogenicity Island : il fenomeno coinvolge altri gruppi di geni → Genomic Island



Geni di virulenza

Pathogenicity island
Uropathogenic *E.coli*

Geni per la simbiosi

Symbiosis Island
Mesorhizobium loti

Geni per l'utilizzazione di zuccheri

Metabolic Island
Salmonella senftenberg

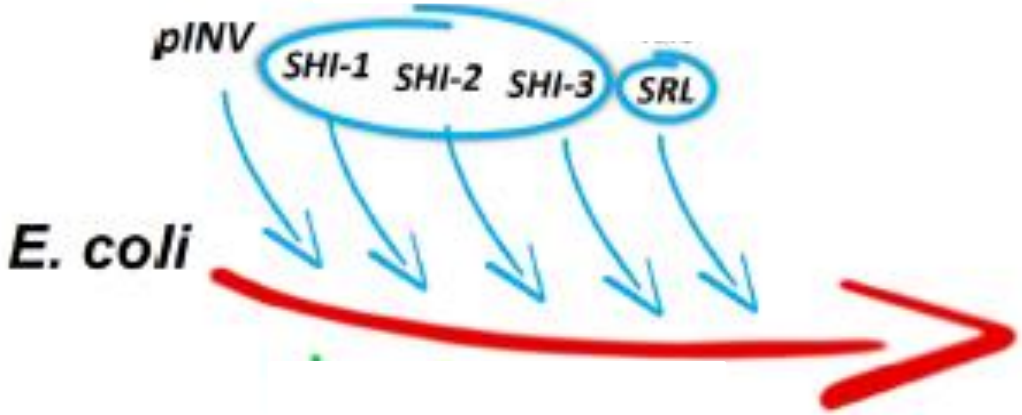
Geni per la resistenza ad uno o più antibiotici

Resistance Island
Staphylococcus aureus

Caratteristiche generali delle Isole genomiche

- Sono regioni di DNA acquisite tramite HGT inserite nelle vicinanze dei **tRNA.**
- Sono fiancheggiate da **DR**
- Contengono diversi geni coinvolti nel processo di adattamento
- Contengono elementi IS funzionali o difettivi
- contiene geni legati alla motilità
- Ruolo fondamentale svolto dal gene **INT** che codifica per l'**INTEGRASI** coinvolta nell'inserzione e delezione di regioni di DNA fiancheggiate da DR

Ruolo delle Isole di patogenicità



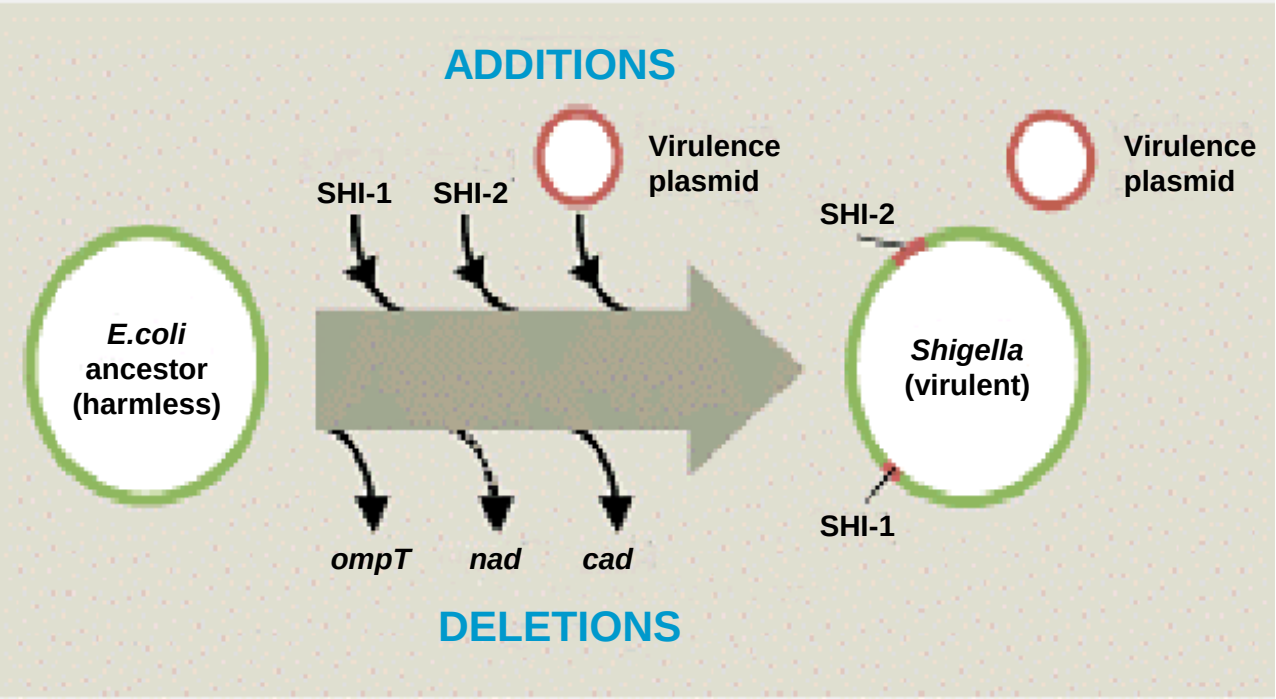
SHI-1: codifica per enterotossina e una proteasi citotossica

SHI-2 e SHI-3: per un sistema di cattura del ferro e sistema di evasione

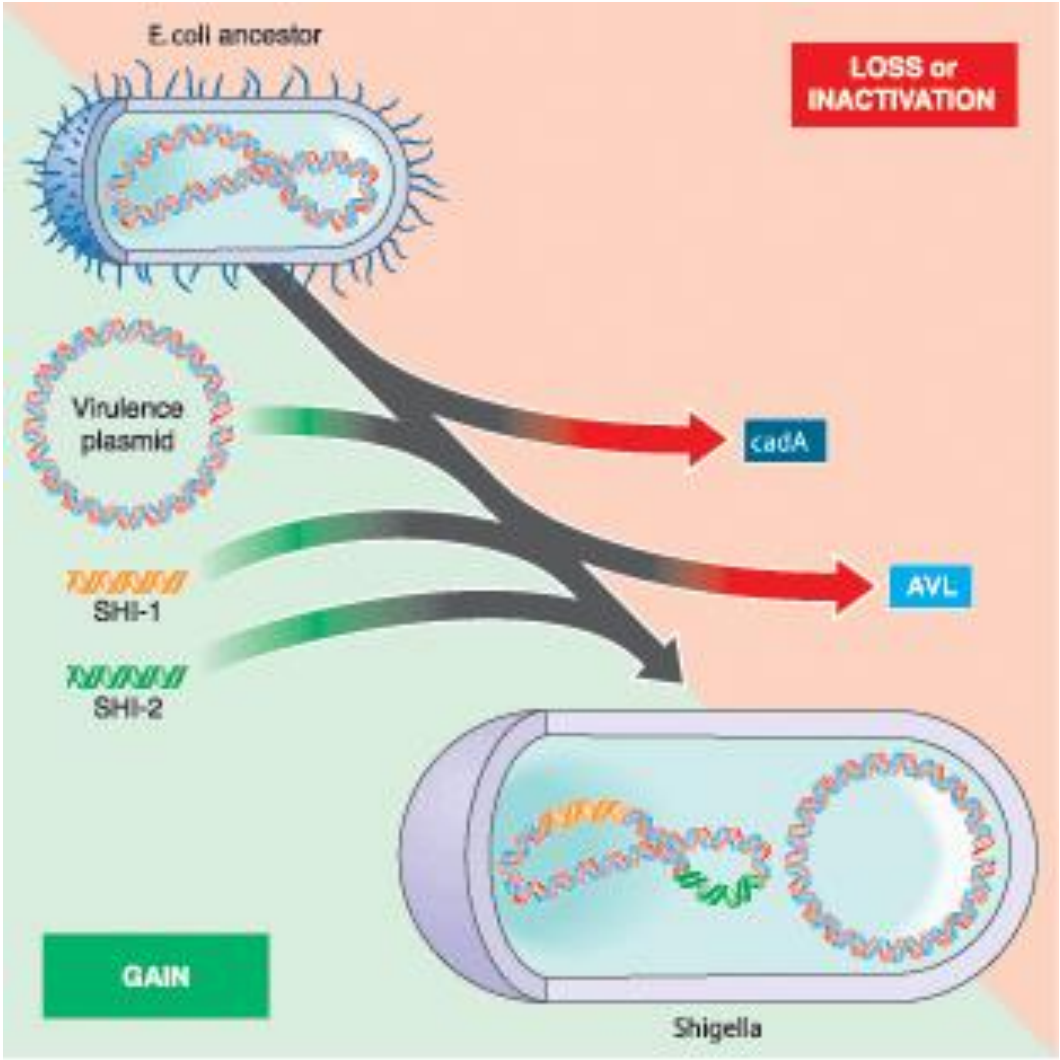
SHI-O: modificazione dell'antigene O (geni fagici)

SRL : resistenza multipla

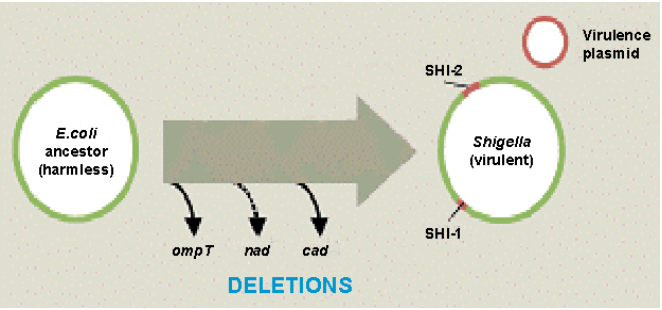
Shedding of genes which interfere with the pathogenic lifestyle *Shigella*



In *Shigella* gene acquisition by horizontal gene transfer is counterbalanced by the loss of native genes, which may have become unnecessary or deleterious for intracellular life.


E.coli ancestor
LOSS or INACTIVATION
Virulence plasmid
cadA
SHI-1
AVL
SHI-2
GAIN
Shigella

Antivirulence genes are eliminated through pathoadaptive mutations

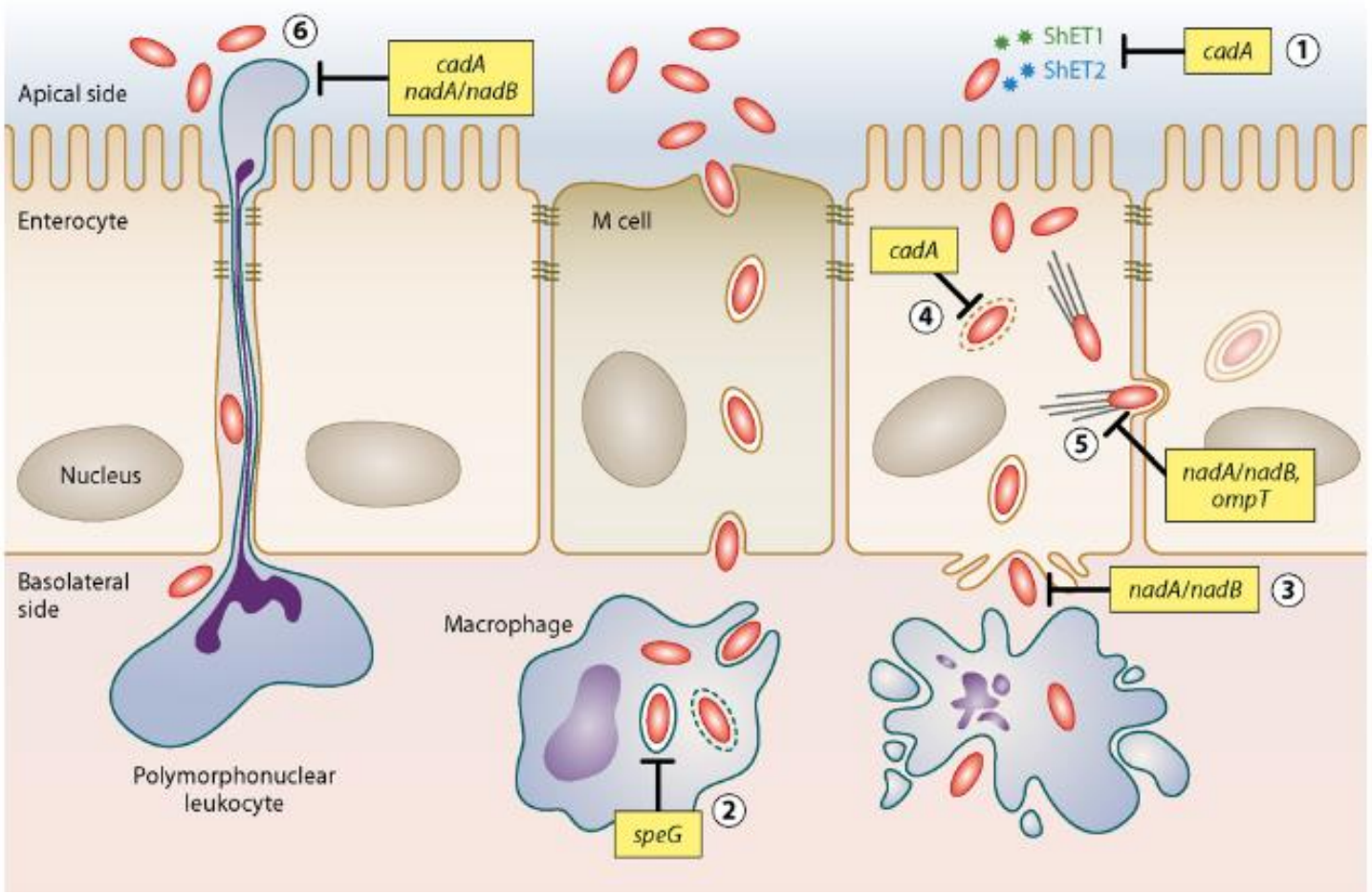


Patho(genicity) adaptive mutations

- improve bacterial fitness in new host environments
- drive a microrganism towards a more pathogenic lifestyle

- Some examples *pathoadaptive* mutations in *Shigella*:
  - loss of cadaverine
  - Loss of acetylspermidine
  - Loss of surface protease OmpT
  - Loss of ability to synthetize nicotinic acid (QUINOLATE)

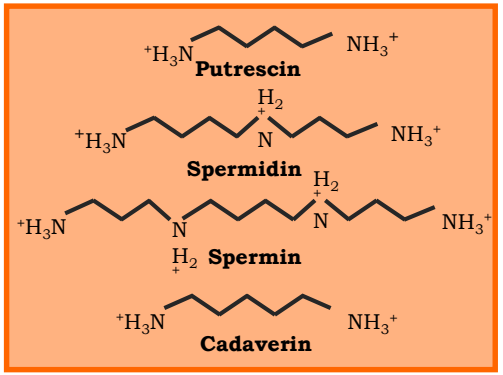
Effect of antivirulence genes of *Shigella* on the invasive process.



Two genetic loci involved in the synthesis of polyamines have been silenced…

Basic functional role of polyamines

Polyamines are small polycationic molecoles present in both, eucaryotic and prokaryotic cells..



They stabilize the plasma membrane and control its permeability

They are involved in response to acid and oxidative stress

They are involved in several processes due to their ability to bind nucleic acids.

A major role is played also in the biosynthesis of proteins:

Polyamines bind to RNA favouring the assembly of the 30S subunit and increasing the fidelity of the translation process

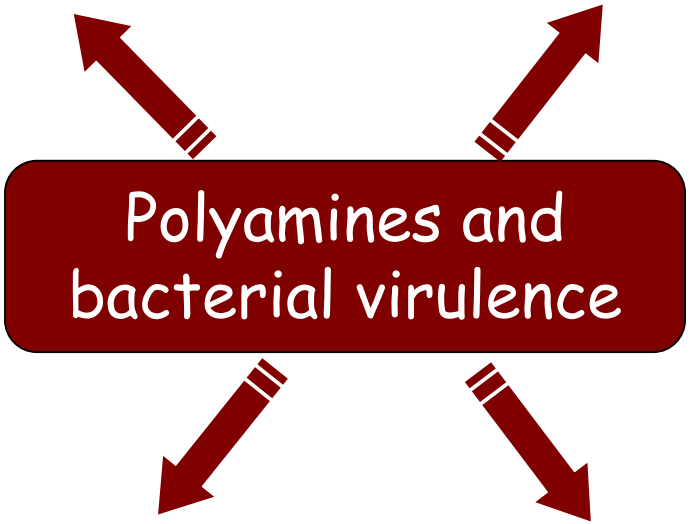
Polyamines exert also a more target-specific action: they affect the translation of several genes, including a number of global regulators, by facilitating the formation of the translation initiation complex

BIOFILM FORMATION

Yersina pestis
Vibrio cholerae
Burkolderia pseudomallei

EXPRESSION OF VIRULENCE

Streptococcus pneumoniae
Shigella/EIEC pathotype



EXPLOITATION OF HOST CELL POLYAMINES

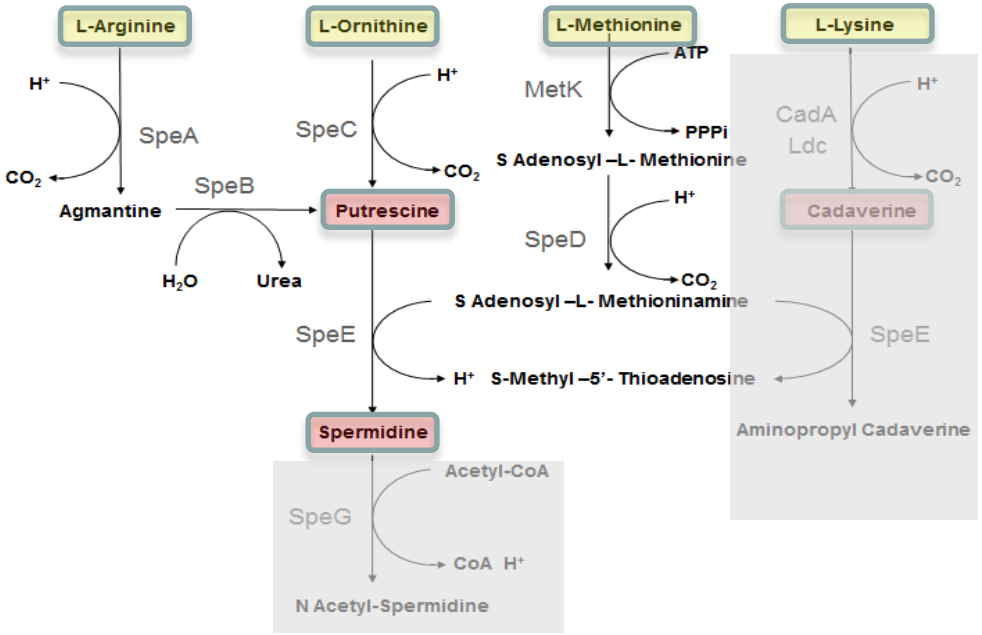
- ***Helicobacter pylori***
  macrophage apoptosis, DNA damages
- ***Legionella pneumophila***
  bacterial intracellular growth
- ***Francisella tularensis***
  disruption of the innate immunity response

EXPRESSION of T3SS

Salmonella Typhimurium (SP_1SP_2)
Pseudomonas aeruginosa (exsCEBA)

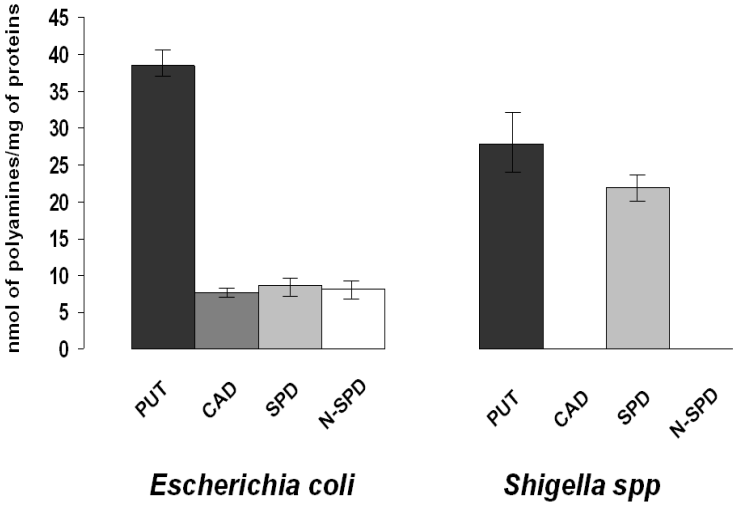
Di Martino et al., 2013

Comparison of the polyamine biosynthesis pathways in *E. coli* and *Shigella* reveals strong differences

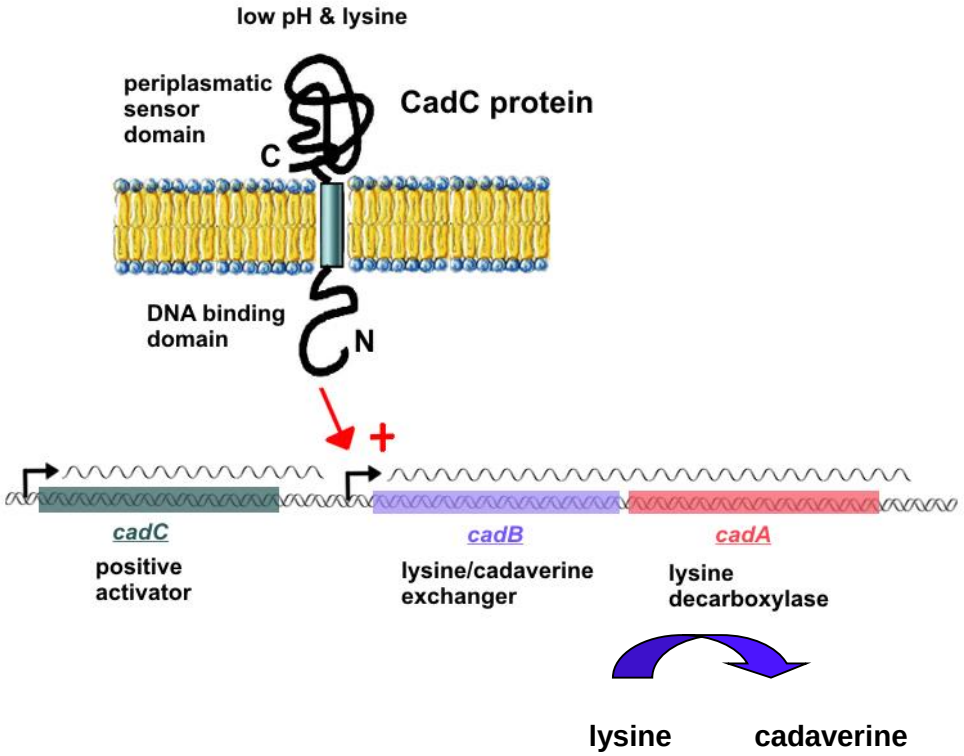


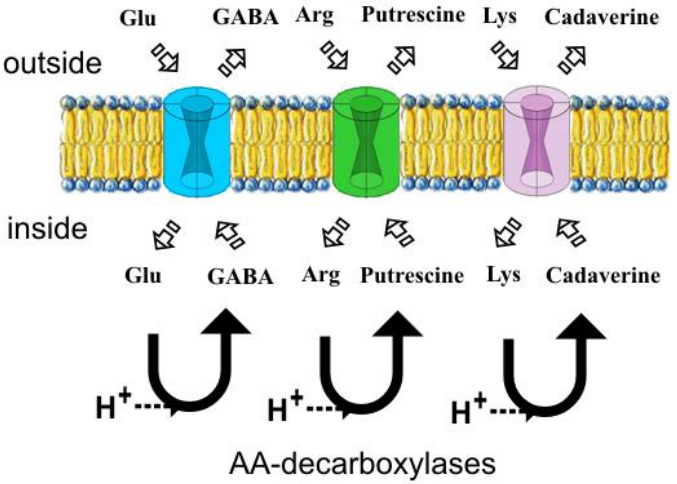
How do the changes in the polyamine pattern influence the *Shigella* invasive process?

Cadaverine and acetylspermidine are lost while spermidine accumulates



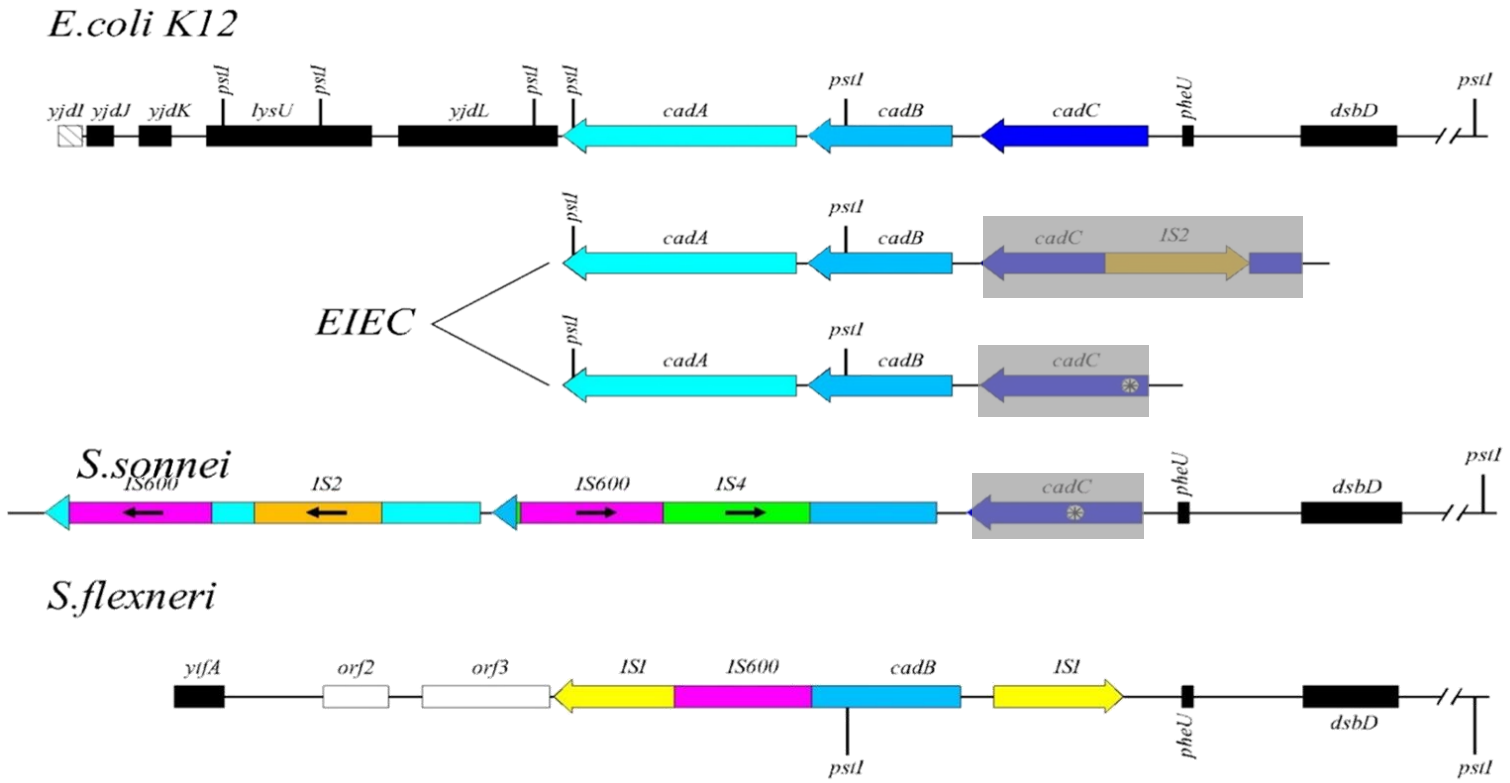
Through lysine decarboxylation at low pH CadA synthesizes cadaverine, a small polyamine





At low pH the release of cadaverine protects the cell from acidification

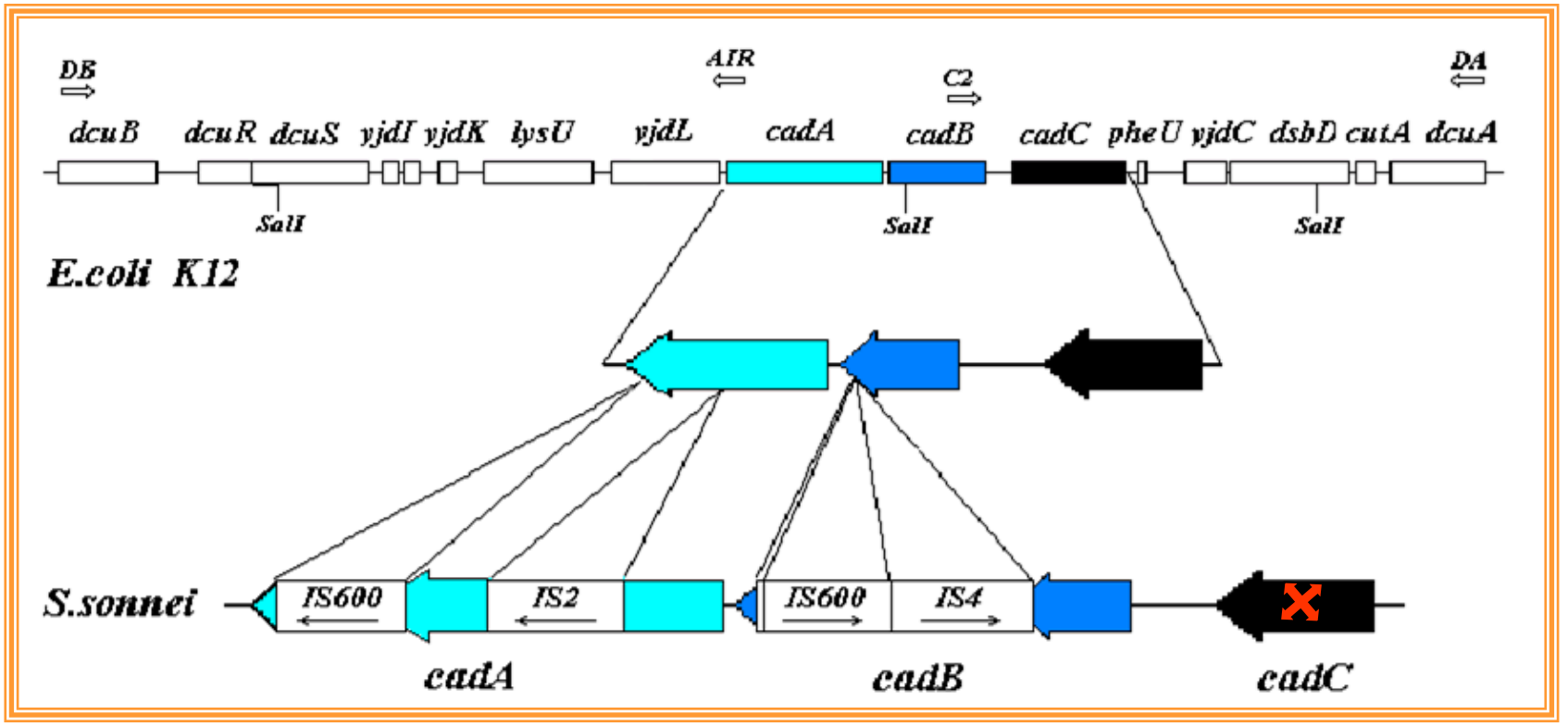
In *Shigella* /EIEC the lack of cadaverine synthesis is obtained through a convergent evolution



Convergent evolution : different strategies , one goal

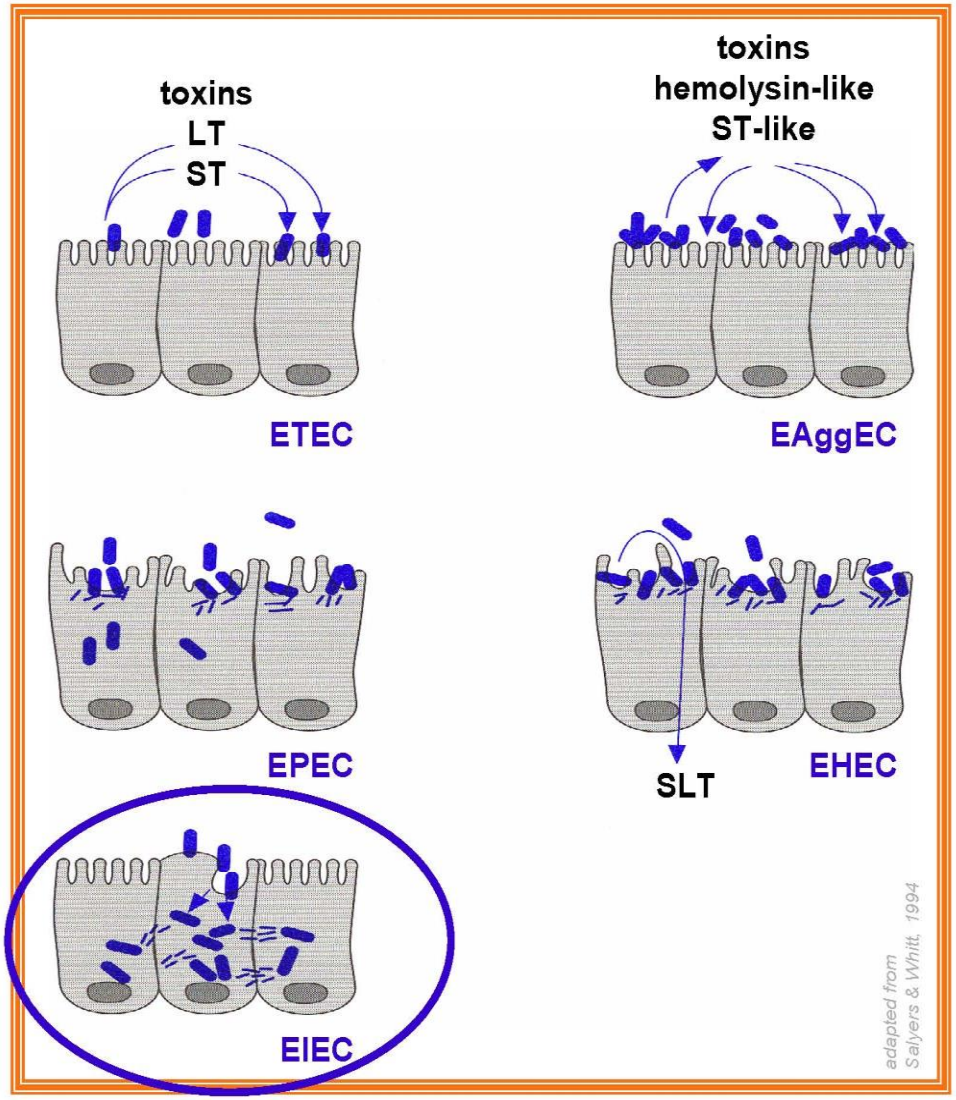
but….the cadC regulatory gene is the preferential target of convergent evolution toward the LCD- phenotype

Shigella sonnei is a new emergent pathogen, often associated with shigellosis in industrial countries



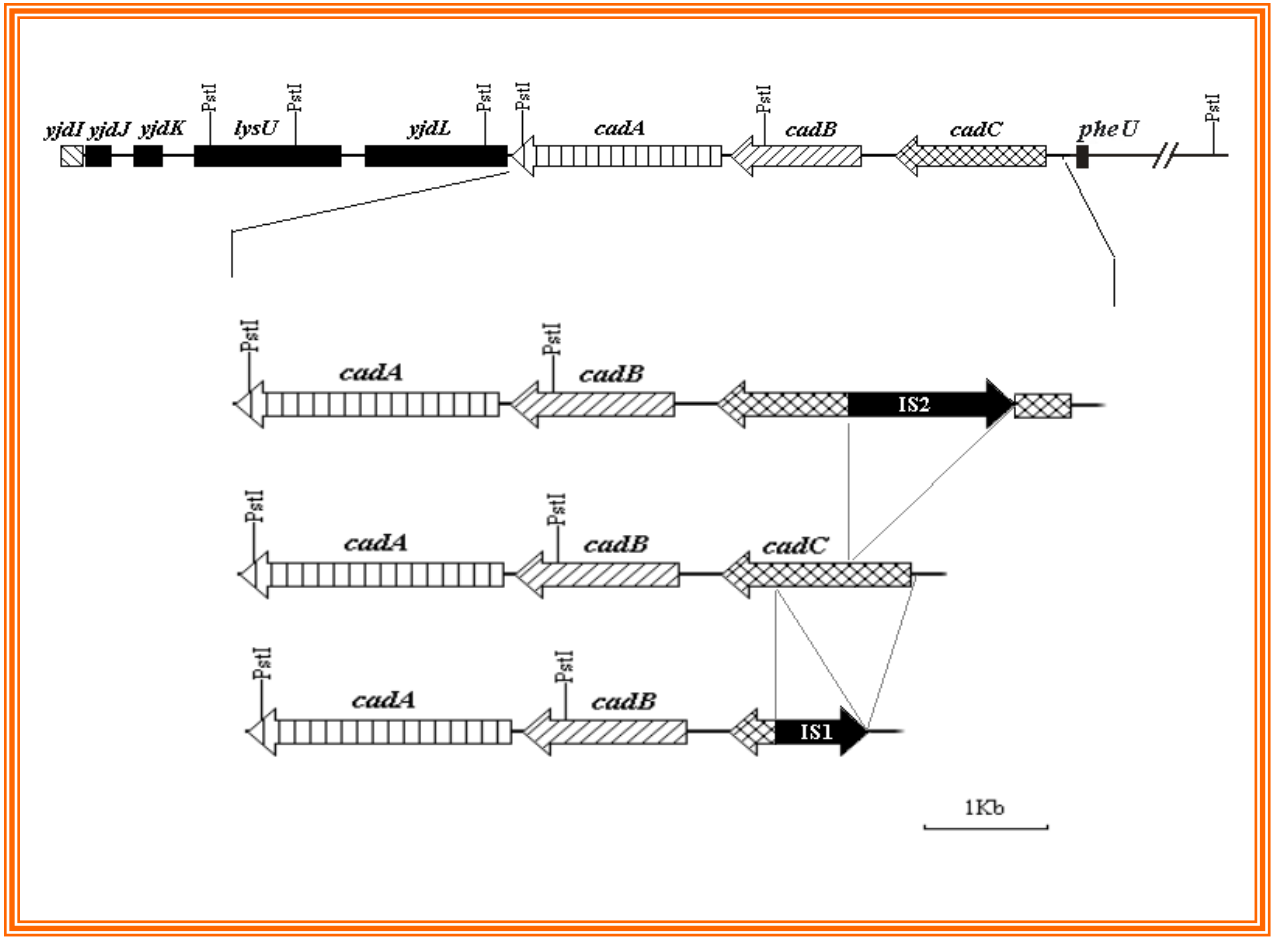
IS sequences have inactivated the *cadBA* genes without inducing deletions. Colinearity with the *E.coli* K12 chromosome is maintained.

Among *E.coli* strains causing intestinal disease all EIEC are unable to synthesize cadaverine

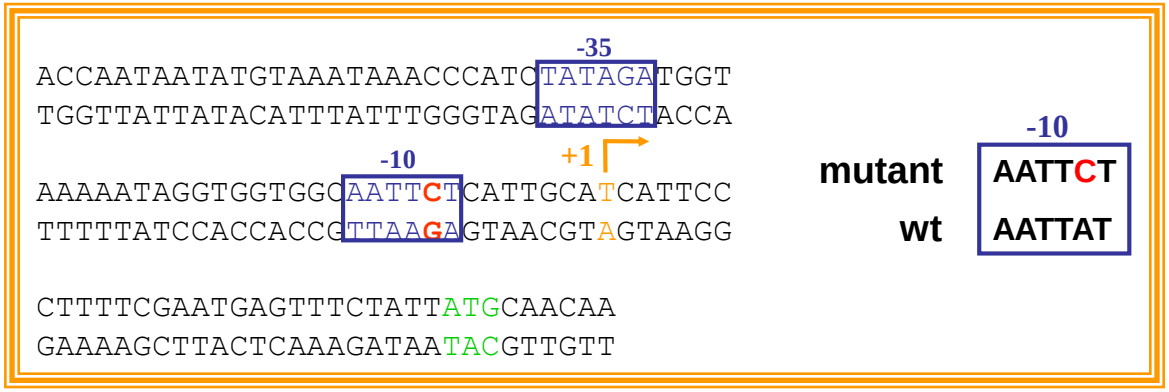


EIEC strains share with *Shigella* the same pathogenicity process, but they exhibit a higher metabolic activity since they retain the ability to catabolize substrates widely used by *E.coli*

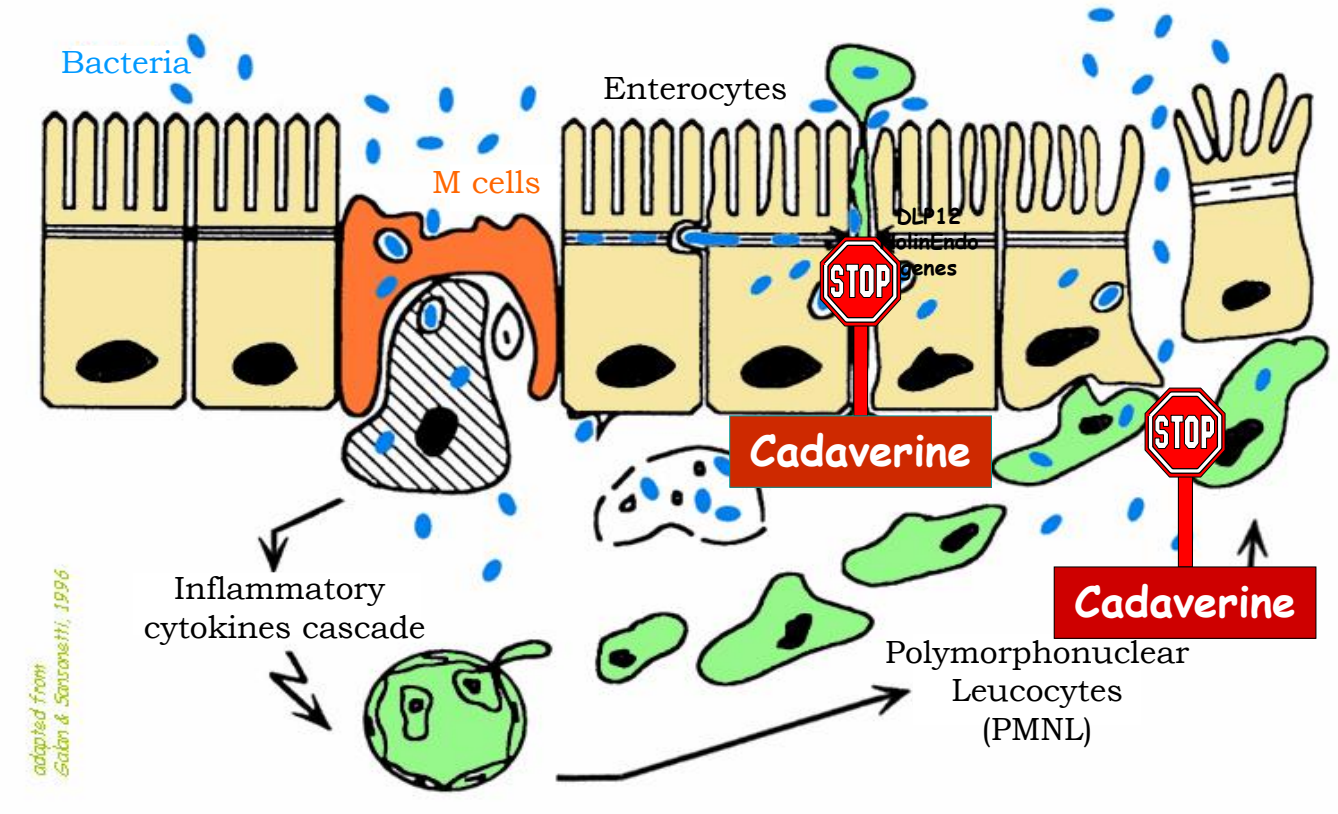
Lack of cadaverine activity in *E.coli* EIEC is induced by IS sequence insertions into the regulatory gene *cadC*



Silencing of the *cadC* gene is obtained by different strategies: in one strain a single point mutation in the promoter region abolishes *cadC* expression



Why is the loss of cadaverine a pathoadaptative mutation?

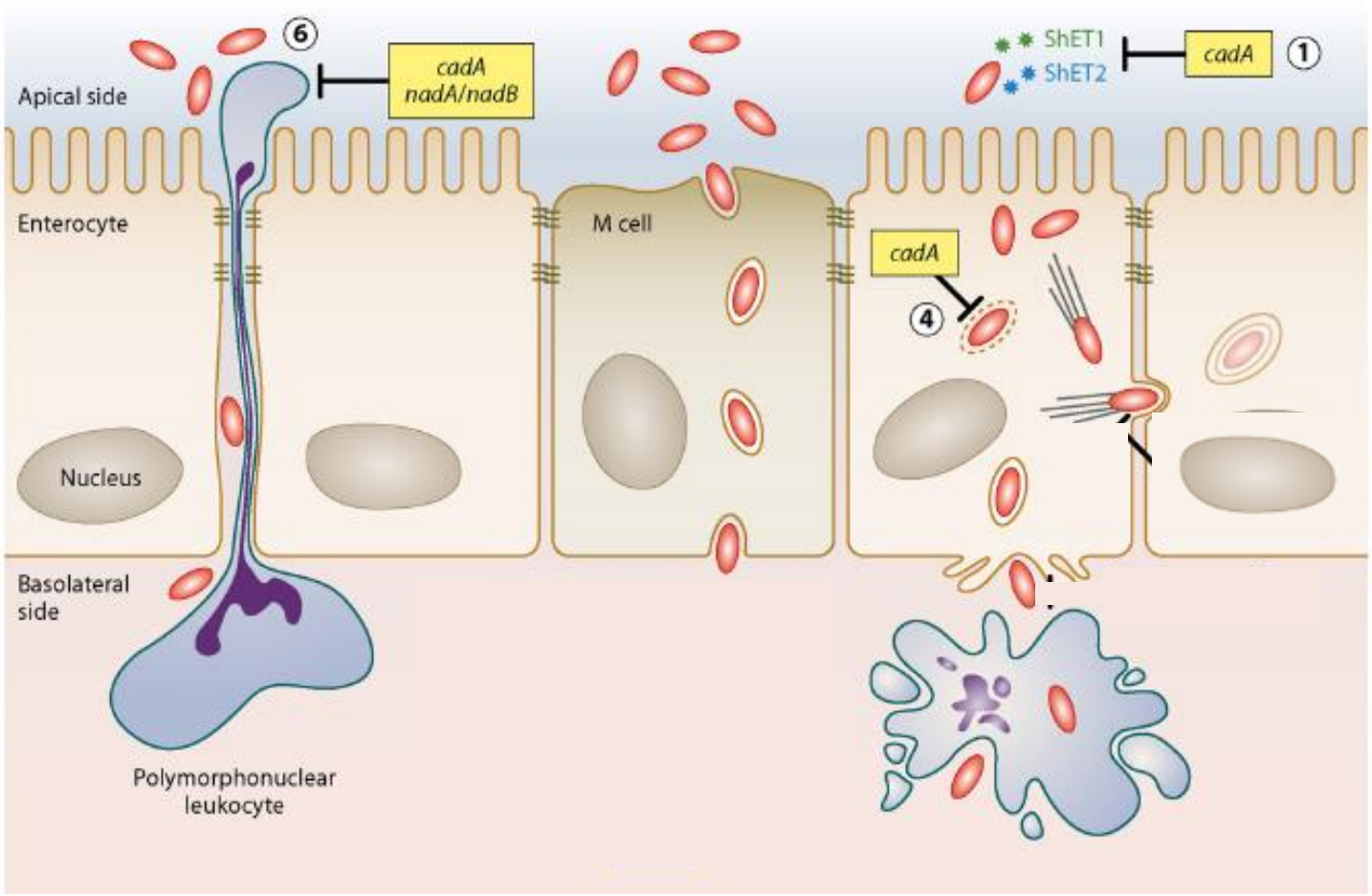


The *Shigella* invasive process is attenuated by cadaverine through:

- retarding the lysis of *Shigella*-containing vacuoles
- decreasing the ability of PMNL to transmigrate
- inhibiting ShEt1 and ShEt2 enterotoxin activity

Cadaverine induces:

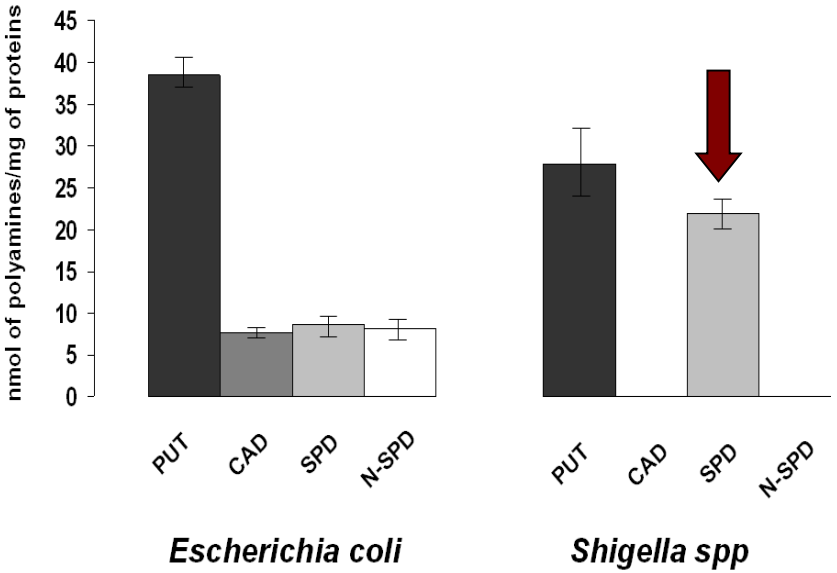
- Endosomal membrane stabilization
- Inhibition of PMN's migration
- Reduction of enterotoxicity



Maurelli et al, PNAS 1998 – Day eta el, IAI 2011

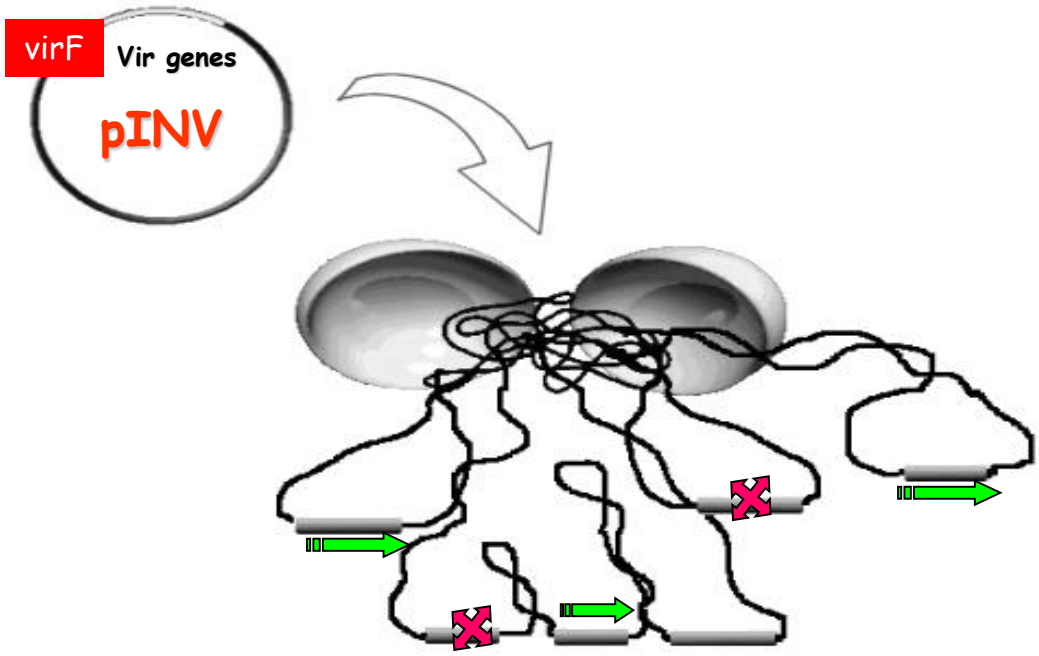
Spermidine accumulation in *Shigella* : a consequence of the acquisition of the VirF regulator?

Besides the loss of cadaverine, the polyamine profile of *Shigella* is characterized by the accumulation of spermidine.



Cadaverine and acetylspermidine are lost while spermidine accumulates

What changes in the transcription profile have been induced by the acquisiton of the plasmid-encoded regulatory factor VirF?



E. coli K12 transcriptome analysis in the presence/absence of VirF shows that VirF-regulated genes can be grouped into…

… genes up-regulated by VirF and **conserved** in *Shigella* …

Gene	VirF induction (x-fold)	Description
htpG	**10.9**	**Heat shock chaperone**
trpA	**7.1**	**Tryptophan synthase**
groS	**6.2**	**GroESL small subunit**
groL	**6.1**	**GroESL large subunit**
bfr	**5.8**	**Bacterioferritin**
prmB	**4.8**	**glutamine methyltransferase**
sucA	**4.5**	**2-oxoglutarate dehydrogenase**
trpS	**3,5**	**Tryptophan-tRNA ligase**
ung	**3,2**	**Uracil-DNA glycosylase**
carA	**3,1**	**Carbamoylphosphate synthase**
agp	**3.1**	**Periplasmic glucose-1-phosphatase**

… and genes up-regulated by VirF and **silenced** in *Shigella* …

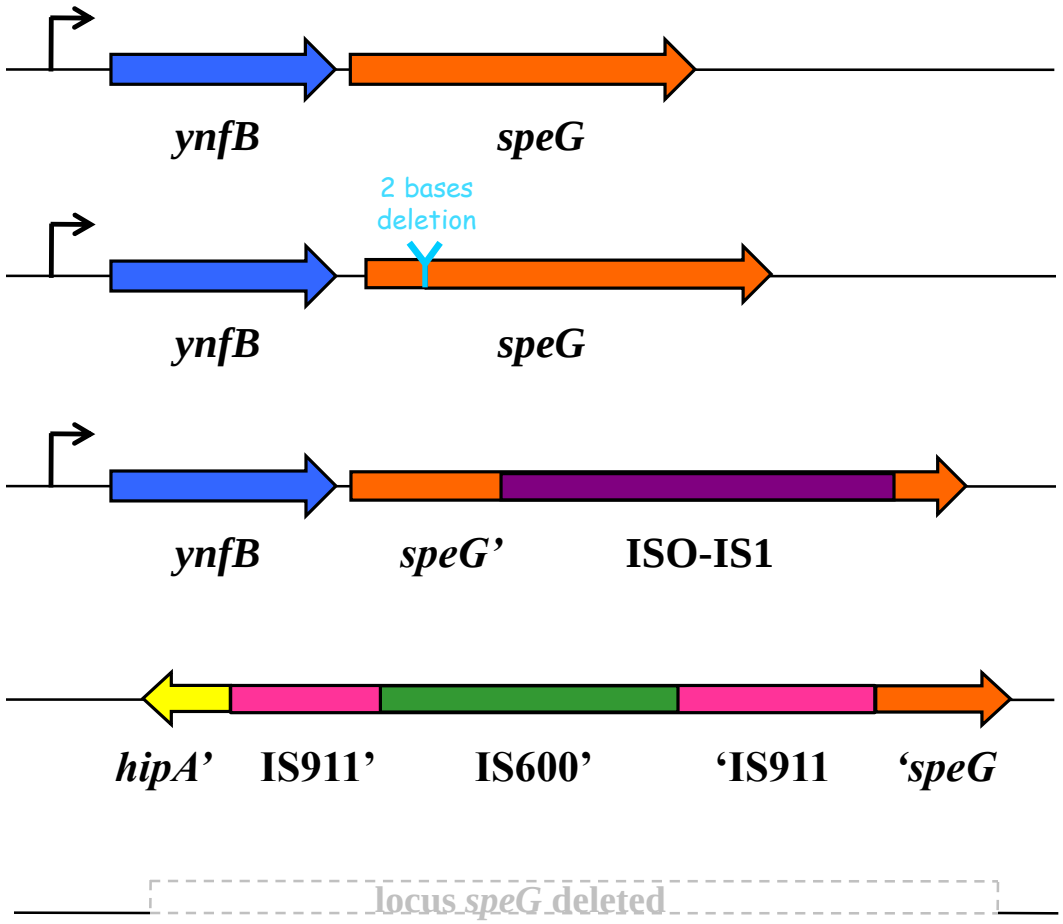
Gene	S.flexneri	S.boydii	S.dysenteriae	S.sonnei
B1172	Δ	Δ	Δ	Δ
paaI	Δ	Δ	Δ	Δ
sgcX	Δ	Δ	Δ	Δ
speG	pseudogene	pseudogene	pseudogene	Δ
yaaX	Δ	Δ	Δ	Δ
yahH	Δ	Δ	Δ	Δ
yaiX	Δ	Δ	Δ	Δ
ybhT	Δ	Δ	Δ	Δ
ycgM	Δ	Δ	Δ	Δ
yfeY	Δ	Δ	Δ	Δ
yghD	Δ	Δ	Δ	Δ
yniB	Δ	Δ	Δ	Δ

speG

- codes for spermidine acetyltransferase
- is up-regulated more than 5-fold in the presence of VirF

Is *speG* inactivation conserved in all *Shigella* species?

Molecular rearrangements of the *speG* locus



***E. coli* K12 MG1655**
1210bp

***S. flexneri* SF301, 803, 8401, 2457T**
1210 bp

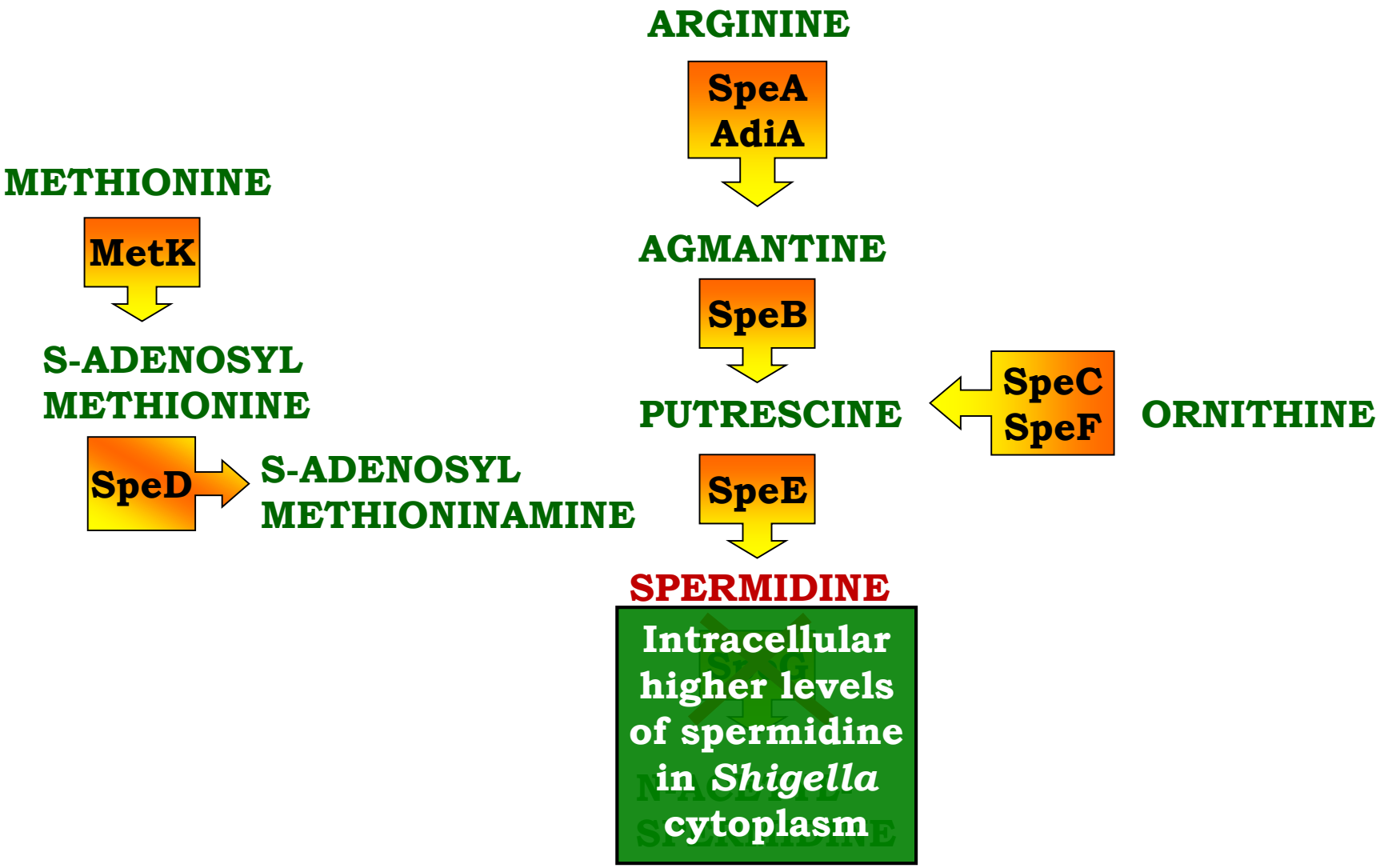
***S. dysenteriae* SD197**
1984 bp

***S. boydii* SB227**
1609 bp

***S. sonnei* SS046**

Convergent Evolution

Spermidine Metabolism



What is the effect of *speG* inactivation on *Shigella* fitness?

To answer this question we performed in vivo assays using derivatives of *S. flexneri* strain M90T:

deleted strain

M90T *speE*
defective *speE* gene which is unable to synthetize spermidine

PUTRESCINE

SPERMIDINE

complemented strains

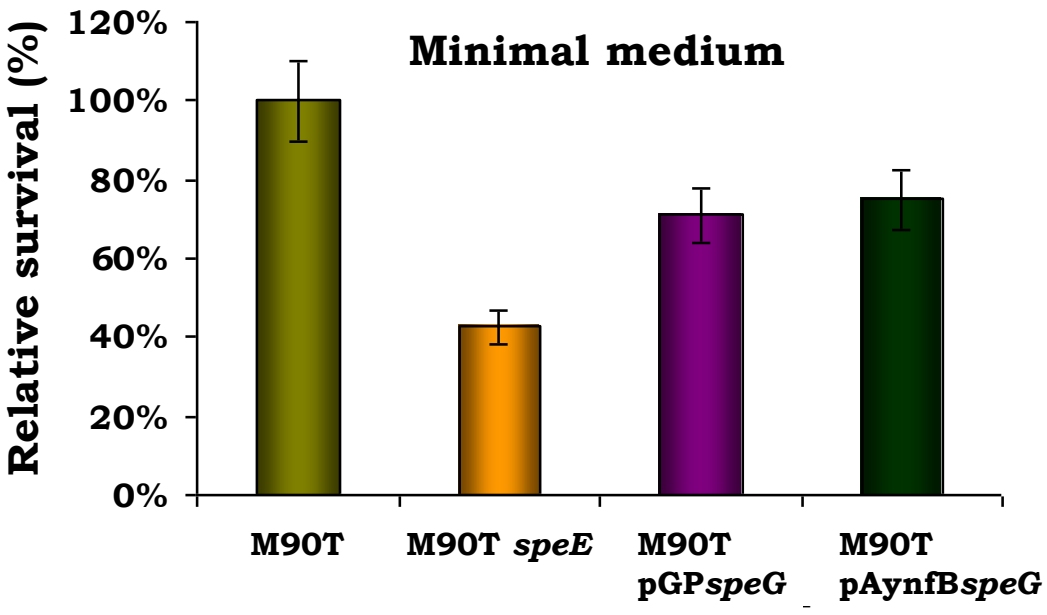
M90T pGP*speG*
with functional *speG* gene under control of inducible promoter *P*tac

M90T pACYC*speG*
with the entire functional *ynfB-speG* operon with its own promoter

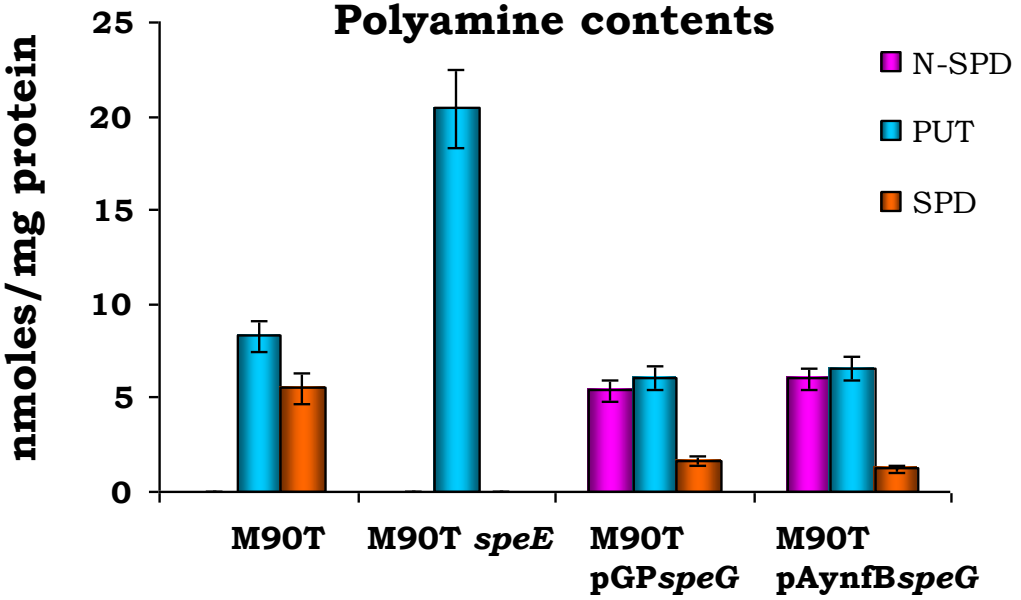
SPERMIDINE

N-ACETYL-SPERMIDINE

Spermidine accumulation increases resistance to oxidative stress



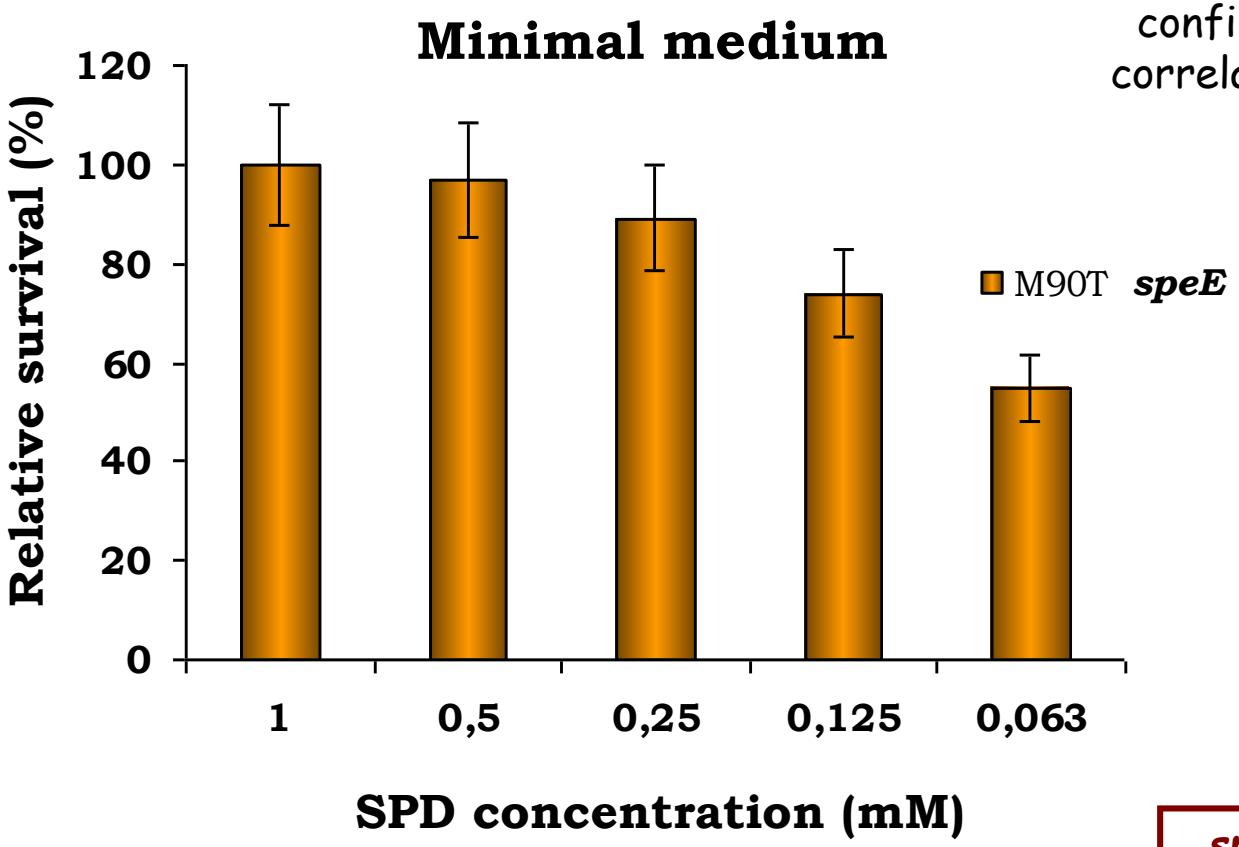
Oxidative stress assay with H_2O_2 (5mM, 30min) on *S. flexneri* M90T reveals that the presence of *speG* reduces resistance to oxidative stress



A reduced resistance to oxidative stress is paralleled by a decrease of intracellular spermidine

Correlation between spermidine and oxidative stress

Oxidative stress assay with H_2O_2 on strains unable to synthesize spermidine (*speE* defective) confirms that survival directly correlates with spermidine (SPD) concentration



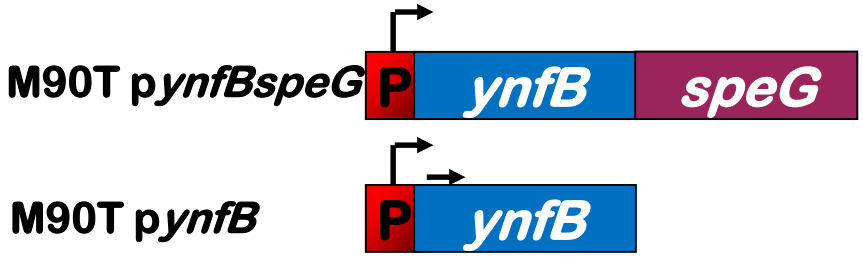
***speG* inactivation improves *Shigella* fitness against oxidative stress**

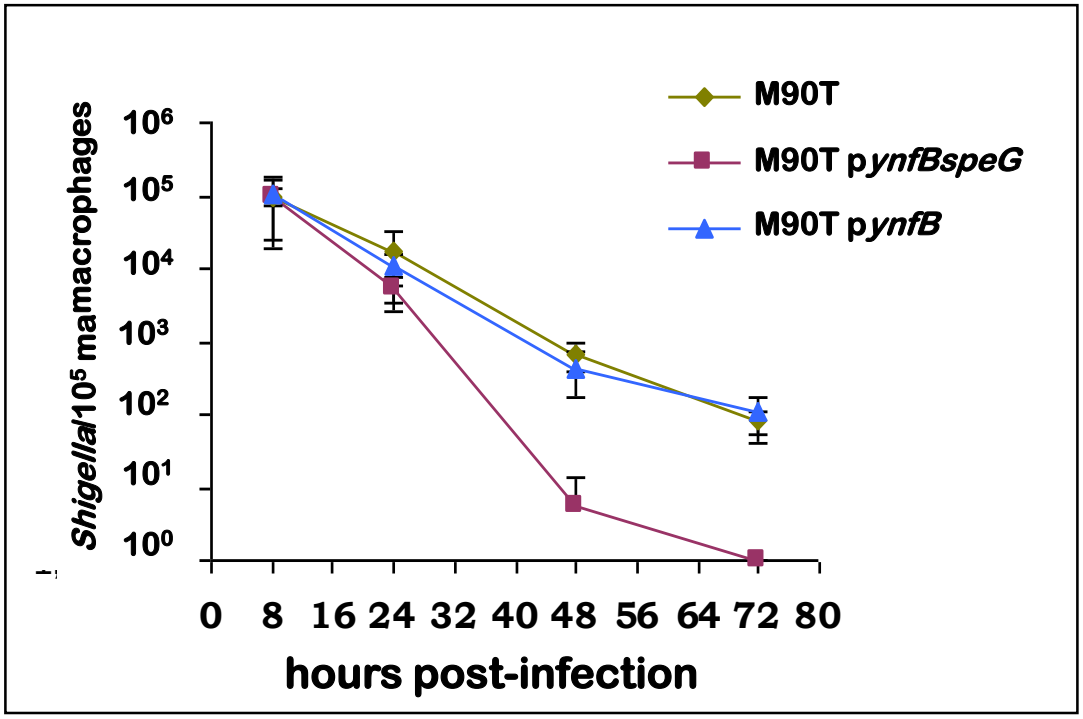
… so, *speG* inactivation improves the fitness of *Shigella* against environmental stresses …

… but does *speG* inactivation improve the fitness of *Shigella* also inside the host?

Intracellular survival of *S. flexneri*

Intraperitoneal injection in BALB/c mice



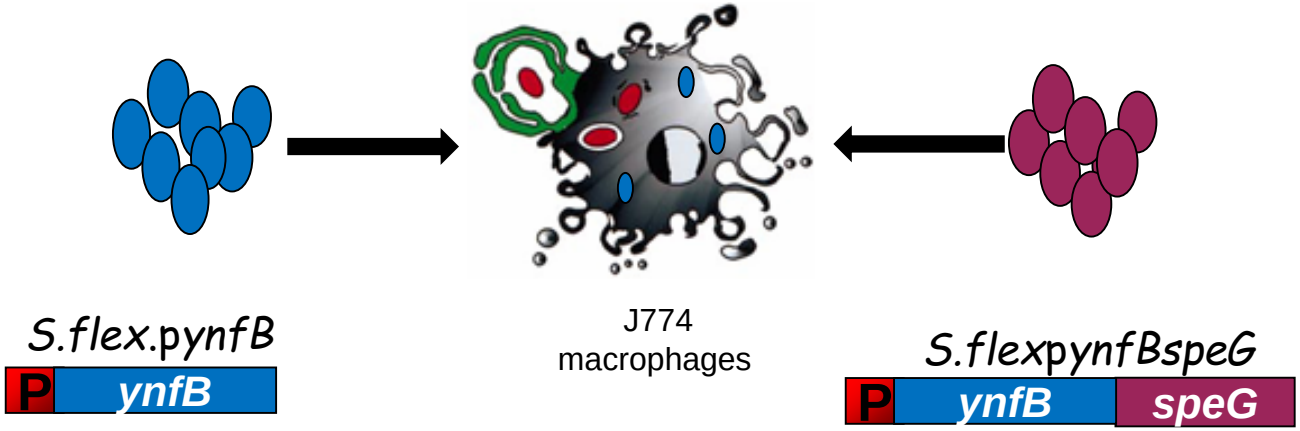


P< 0,04

The introduction of the *speG* gene into *Shigella* reduces bacterial survival within macrophages

in collaboration with Prof. Maurizio Sanguinetti

Competitive infection between *S. flexneri* strains



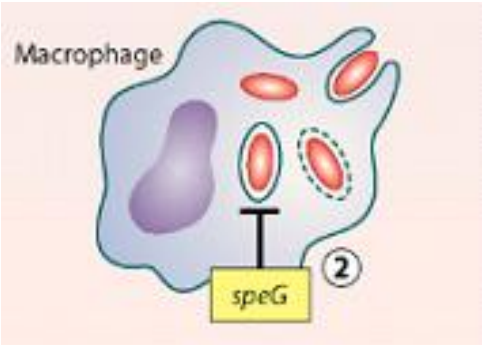
$$C.I. = \frac{S.\ flex + P\ ynfB\ speG}{S.\ flex + P\ ynfB}$$

(Competitive Index)

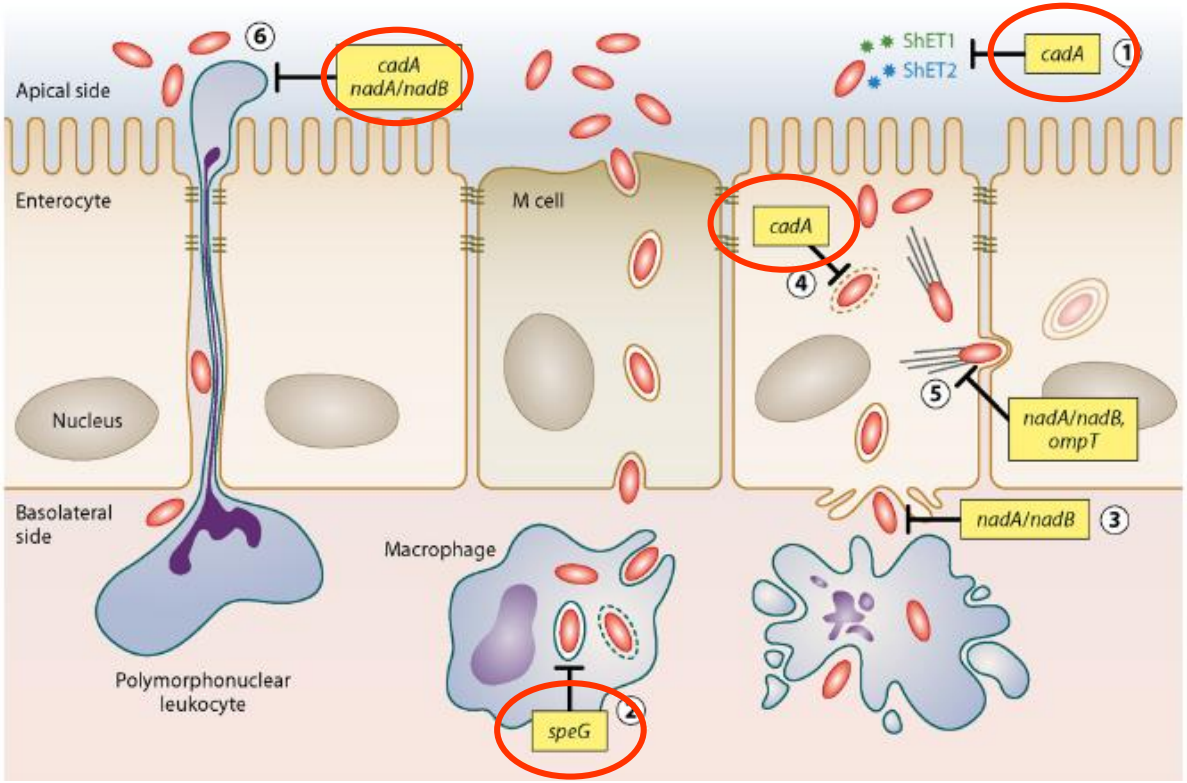
0.7 (1h)

0.4 (2h)

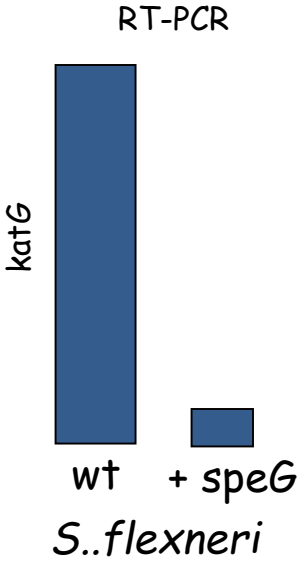
speG inactivation improves the survival within the macrophages



Effect of polyamines to the *Shigella* invasive process



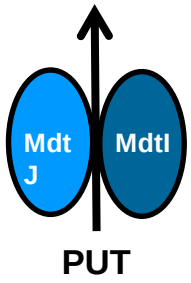
speG inactivation induces
8-fold overexpression of *katG*
encoding hydroperoxidase I

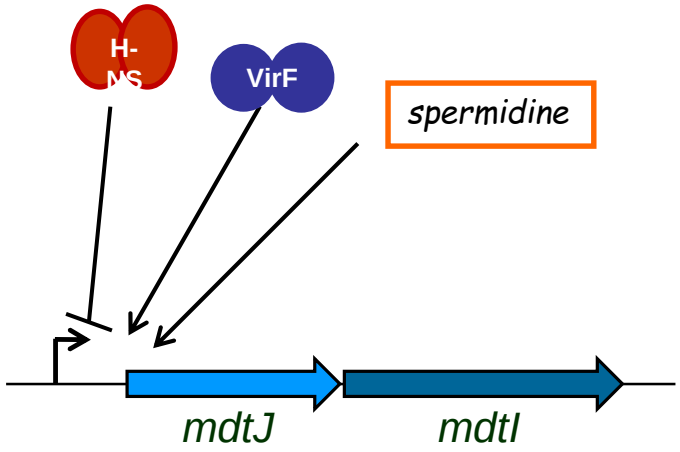


Acquisition of VirF

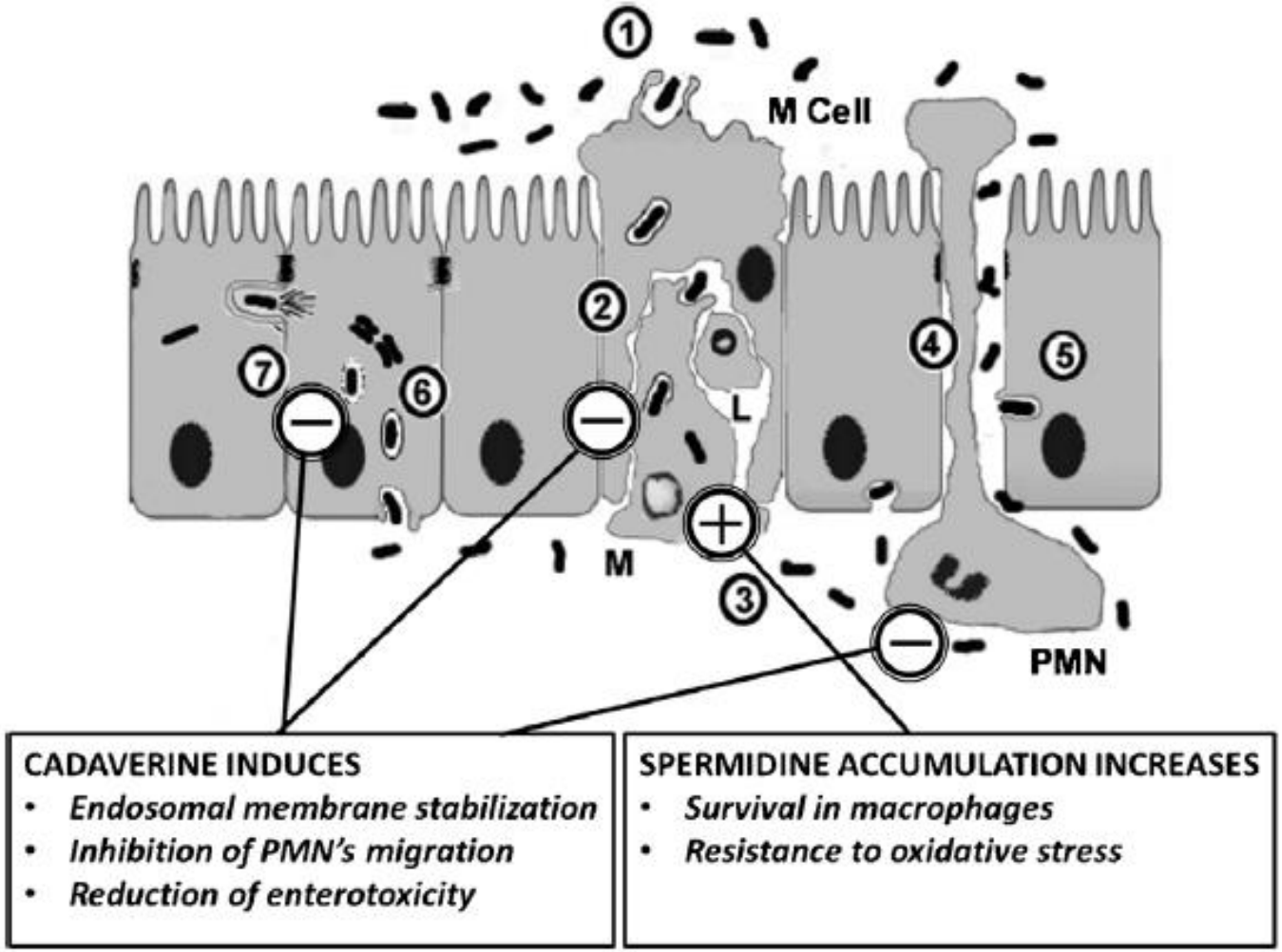
- Activation of the plasmid virulence genes as a function of temperature
- Up-regulation of several genetic systems, some of which are probably involved in increasing the pathogenicity potential of the ancestral strain
- Deletion of genes whose up-regulation has a deleterious effect on cell survival or on the establishment of a fruitful host-pathogen interaction
- Activation of genes involved in the survival of *Shigella* in the presence of high spermidine level

VirF is able to activate the MtdJI efflux pump, which secretes putrescine, the precursor of spermidine.





Effect of polyamines on the *Shigella* invasive process



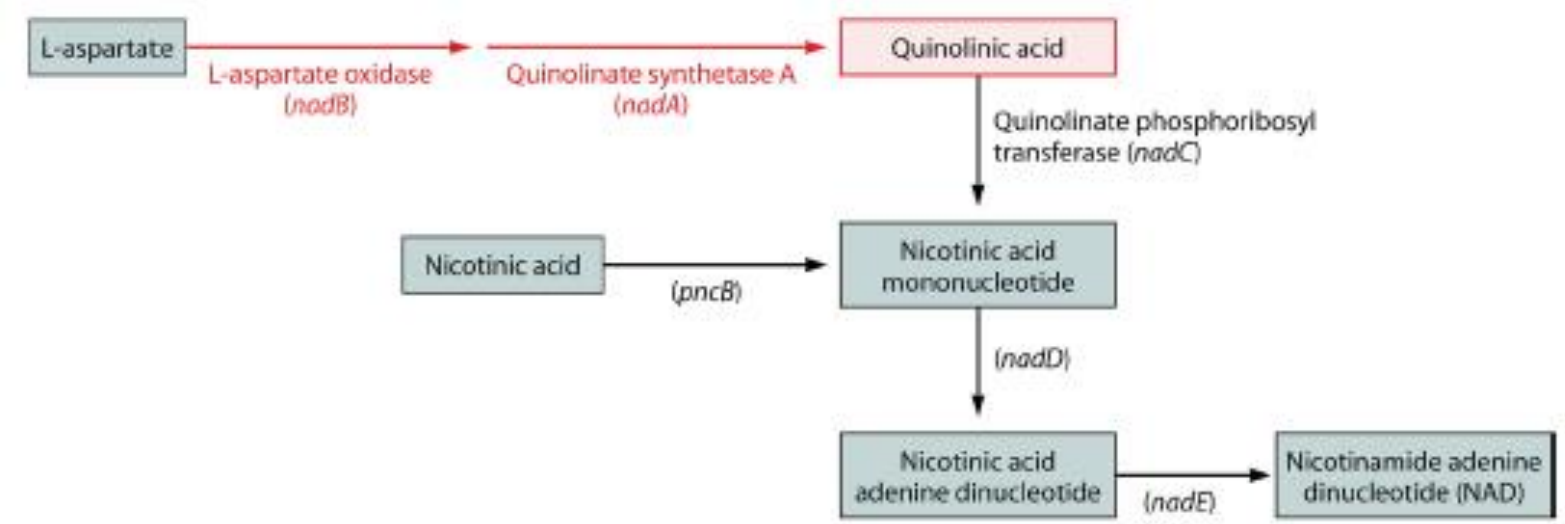
Acquisition of plasmid virF regulator

- Activation of the plasmid virulence genes as a function of temperature
- Up-regulation of several genetic systems, some of which are probably involved in increasing the pathogenicity potential of the ancestral strain
- Deletion of genes whose up-regulation has a deleterious effect on cell survival or on the establishment of a fruitful host-pathogen interaction
- Activation of genes involved in the survival of *Shigella* in the presence of high spermidine level

Mutazioni patoadattative in Shigella

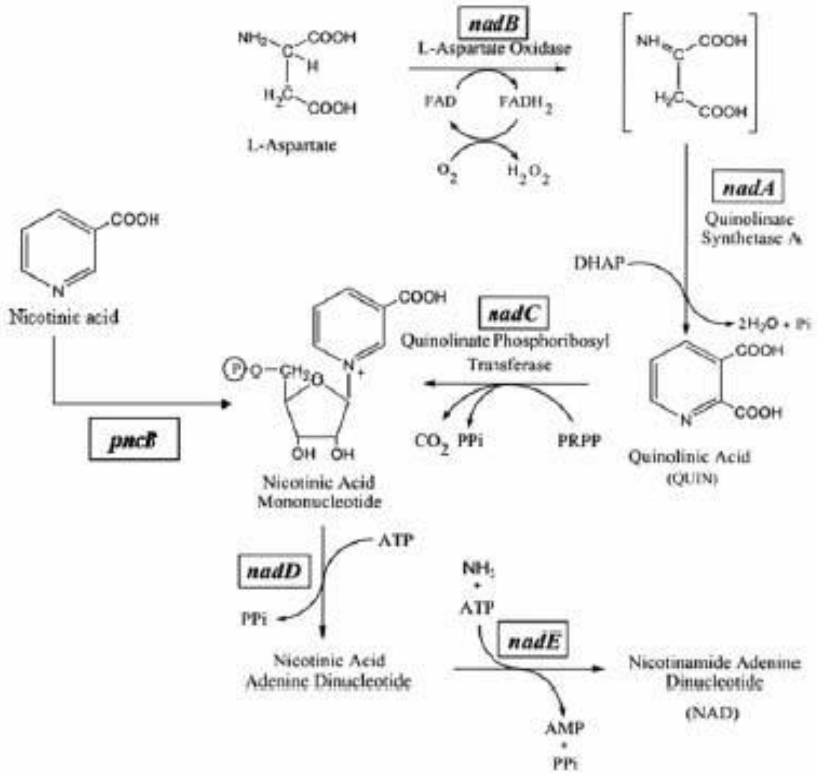
Antivirulence Genes	Biochemical activity	Antivirulence functions	Effects
ompT	Surface protease	Degradation of IcsA outer membrane protein	Inhibition of actin-based intracellular motility
cadA	Lysine decarboxylation	Synthesis of cadaverine	Attenuation of enterotoxicity; inhibition of PMNs migration; prevention of lysis of *Shigella* containing phagocitic vacuole
nadA, nadB	Synthesis of nicotinic Acid	Synthesis of QUIN	Prevention of intercellular spreading; reduction of PNMs migration; inhibition of T3SS-mediated secretion of IpaB and IpaC
speG	Spermidine Acetyl Transferase	Conversion of spermidine to acetyl-spermidine	Increased sensitivity to oxidative stress; reduction of intracellular survival in macrophages
argT	Transport of aminoacids	Not determined	Inhibition of invasion of HeLa cells
flh	Synthesis of flagella	Not determined	Potential activator of host immune system
csg	Synthesis of curli	Not determined	Potential activator of host immune system

Another pathoadaptive mutation: the silencing of *nad* genes involved in the synthesis of nicotinic acid



Quinolic acid (QUIN), the product of the NadA/NadB enzymatic reactions, inhibits both, invasion and intercellular spread of *Shigella*

I geni nad coinvolti nella sintesi dell'acido nicotinico



QUIN

Fig. 1. L-Aspartate-dihydroxyacetone phosphate pathway for *de novo* NAD synthesis in *E. coli* K-12 [adapted from Flachmann *et al.* (1988)].

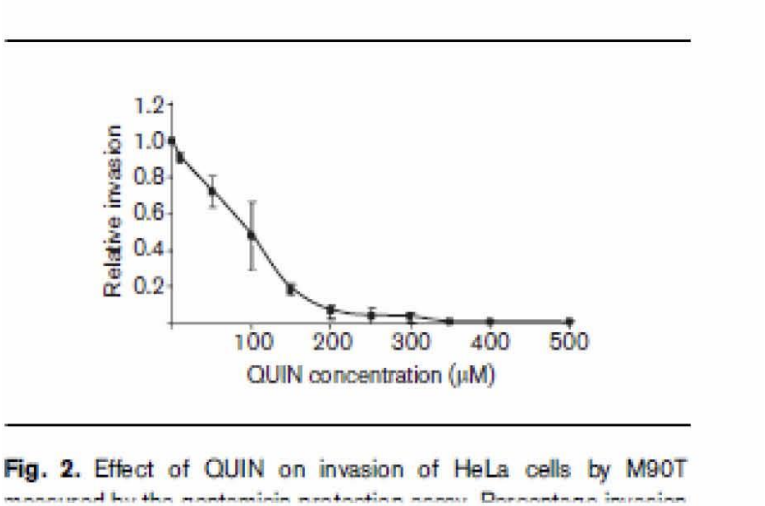
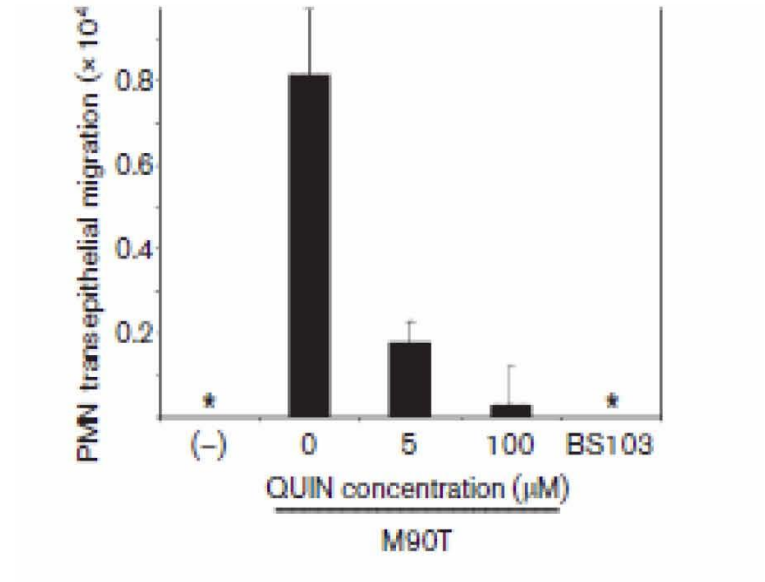


Fig. 2. Effect of QUIN on invasion of HeLa cells by M90T

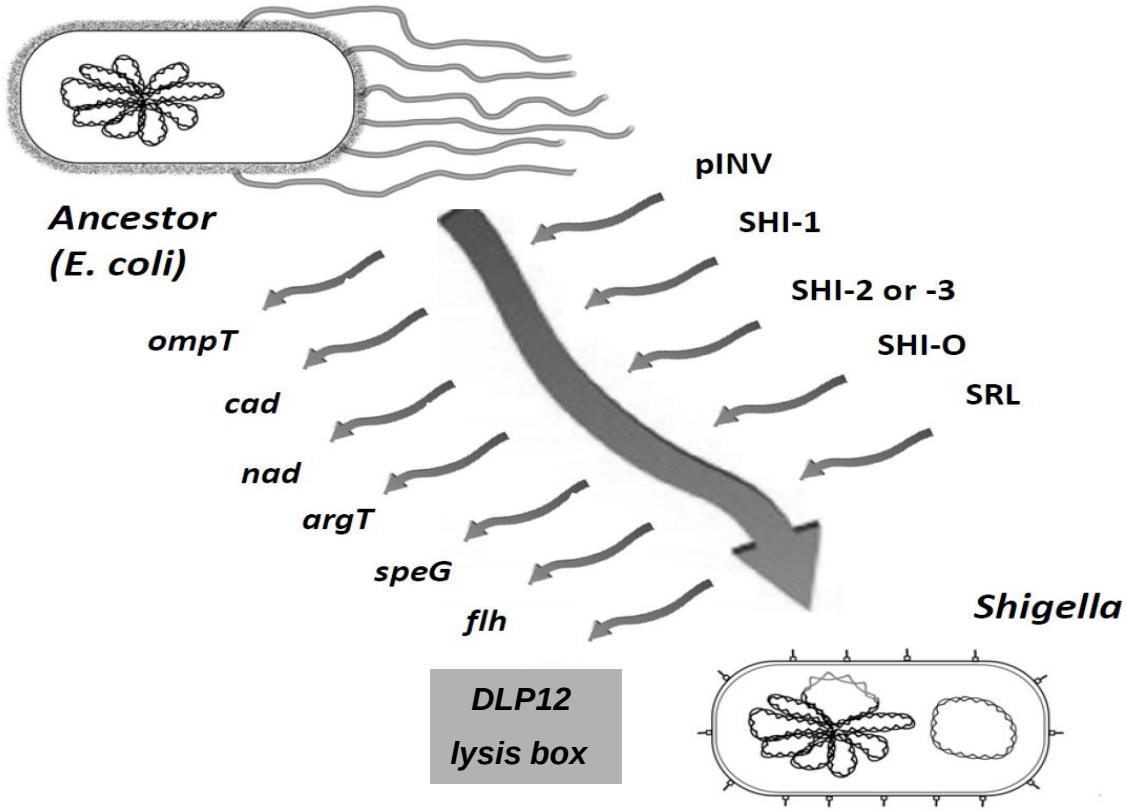


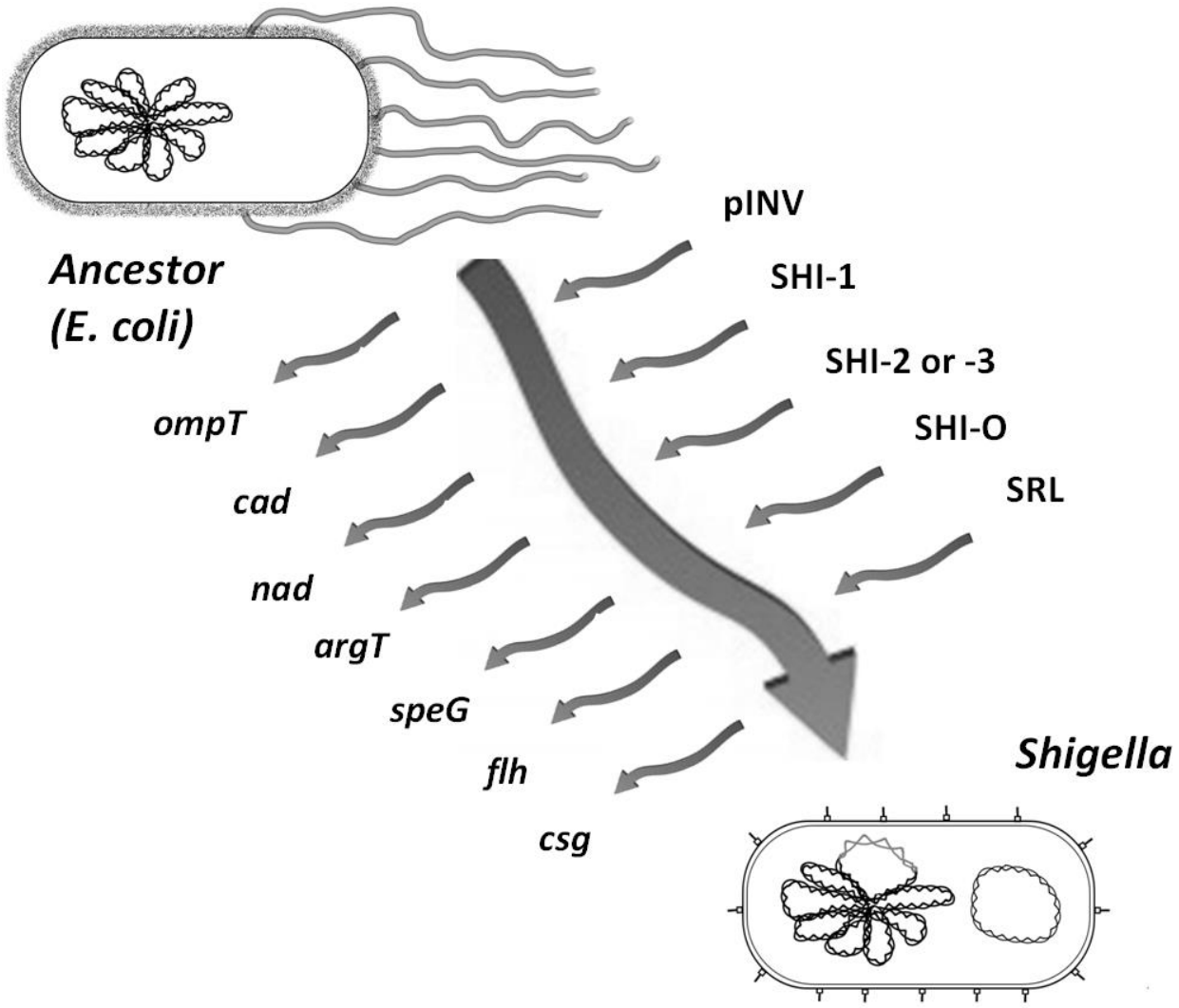
Il QUIN un intermedio nella sintesi dell'acido nicotinico interferisce nel processo di invasività di *Shigella* sia a livello dell'efficienza di invasione che nella trasmigrazione dei PMN verso il sito d'infezione

Examples of genetic rearrangements in the nad BA genes: another case of convergent evolution

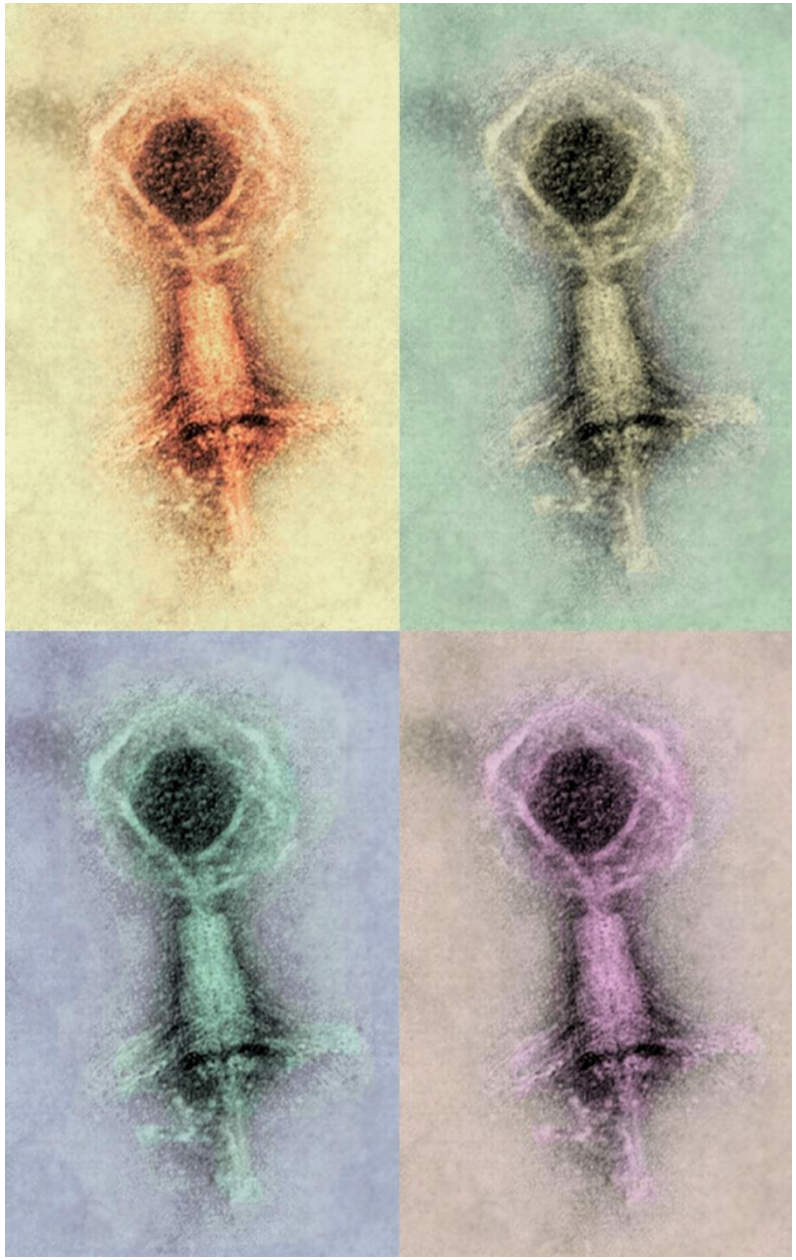
S. flexneri 2457T	2a	3	**A111V**, **C128Y**, **T252A**, **Q271R**, G304D	AF403415	R80H, **Q95P**, (I108V, E141Q, T142S, L149Q), **C354***, (D415G, I416V)	AF403416	4
S. flexneri BS510	3a	3	None	EF473659	**A73S**, (I108V, E141Q, T142S, L149Q), **C354***, (D415G, I416V)	EF473668	CDC
S. flexneri M90T	5a	3	**A111V**, **T252A**	EF473666	R80H, **Q95P**, (I108V E141Q, T142S, L149Q), **C354***, (D415G, I416V)	EF473657	21
S. dysenteriae 197	1	Outlier	R134H, (G191A), **P219L**, R257W	NC_007606	(V167I), **D218N**, (D415G, I416V)	NC_007606	25
S. dysenteriae BS681	8	Outlier	none	EF473662	**G44V**, (I108V, E141Q, L149Q), V180I, (Y412D, D415G, I416V)	EF473671	CDC
S. sonnei BS513	NA[g]	Outlier	IS21 between aa 292 and 293	EF473661	IS*600* in codon 233	EF473670	CDC
E. coli (EIEC) strains							
EDL1284	O124	NA	**G198S**, **V260G**	EF473665	Portion of IS*600* after aa 52; deletion aa 53 to 192	EF473674	7
E. coli (EIEC) strain 1	O136	NA	**G198S**	EF473664	IS*600* between aa 52 and 53	EF473673	L. Trabulsi

The long path of *Shigella* towards pathogenicity may involve more pathoadaptive mutations……



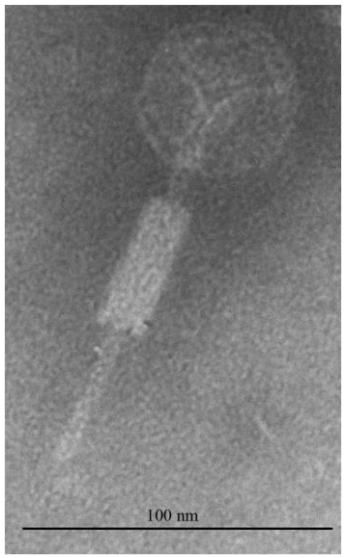

Ancestor
(E. coli)
pINV
SHI-1
SHI-2 or -3
SHI-O
SRL
ompT
cad
nad
argT
speG
flh
csg
Shigella

Phages and virulence or
…….antivirulence?



Gioacchino Micheli

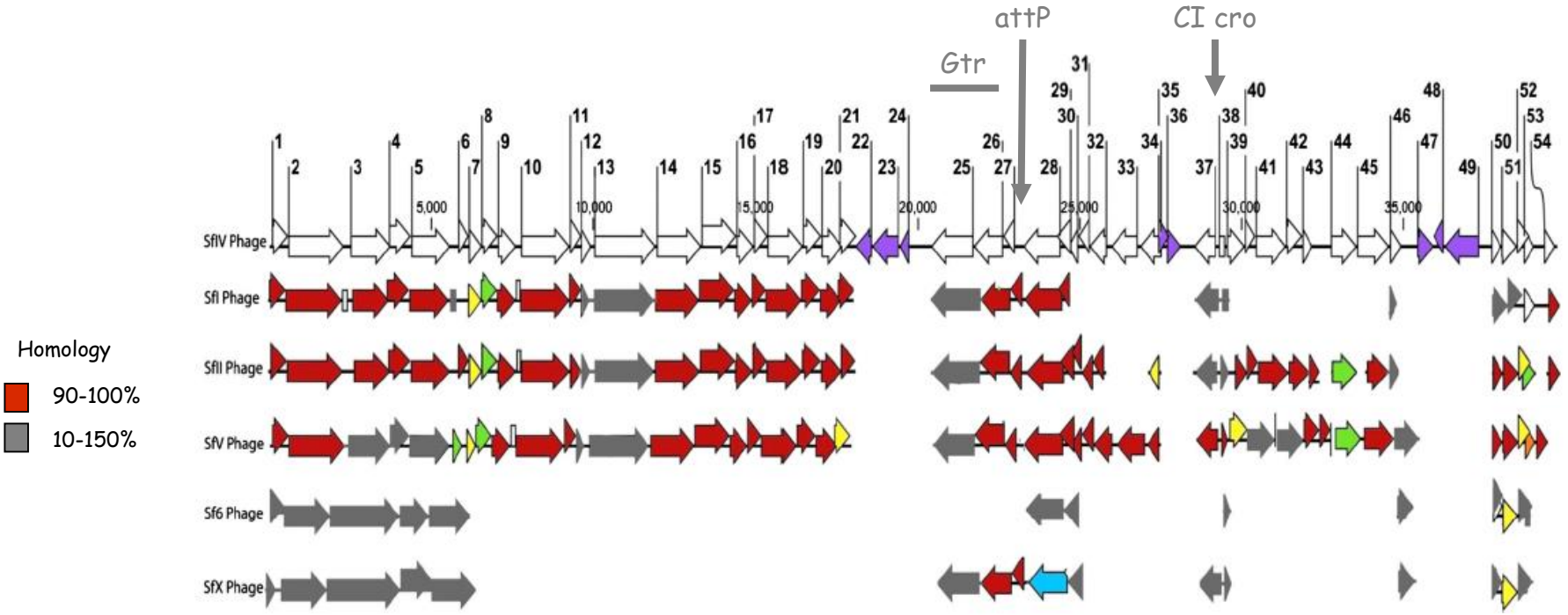
Phages and O-antigen in *Shigella*



SfIV phage

Several seroype-converting phages (Sfl) have been isolated in *Shigella*. They contain genes encoding glucosyltransferase and/or acetyltransferase, responsible for the modification of the O-antigen.

Two genes - gtrA and gtrB - are well conserved. They encode proteins involved in the transfer of the glucosyl group, while the third gene (encoding glucosyltransferase) is serotype-specific.

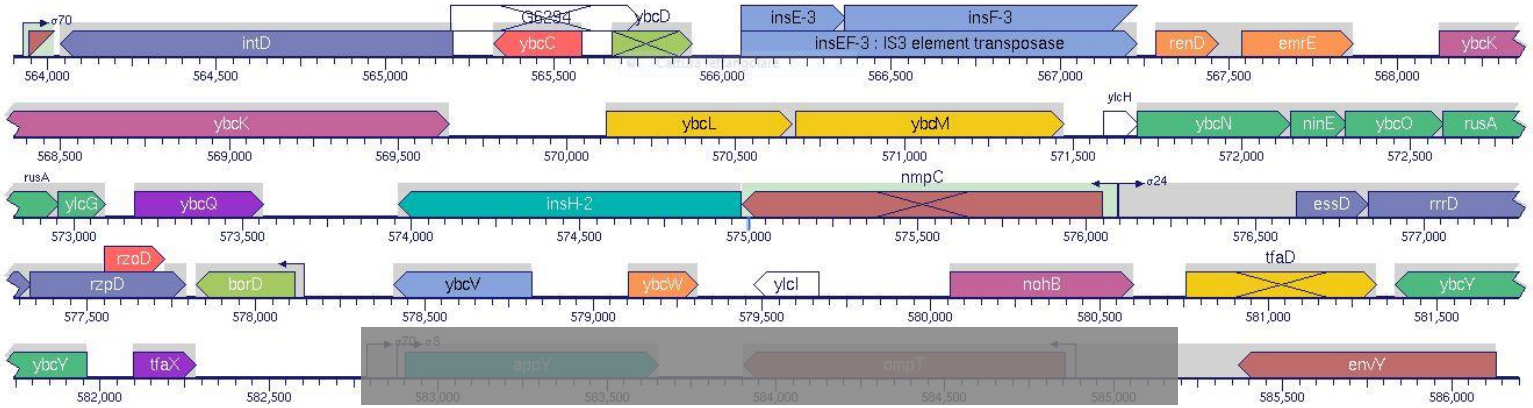


DLP12: an antivirulent prophage?

DLP12 :

- is a **D**efective **L**ambdoid **P**rophage integrated at 12 min in *E. coli* chromosome

Within its genome DLP12 carries the gene encoding the OmpT protease



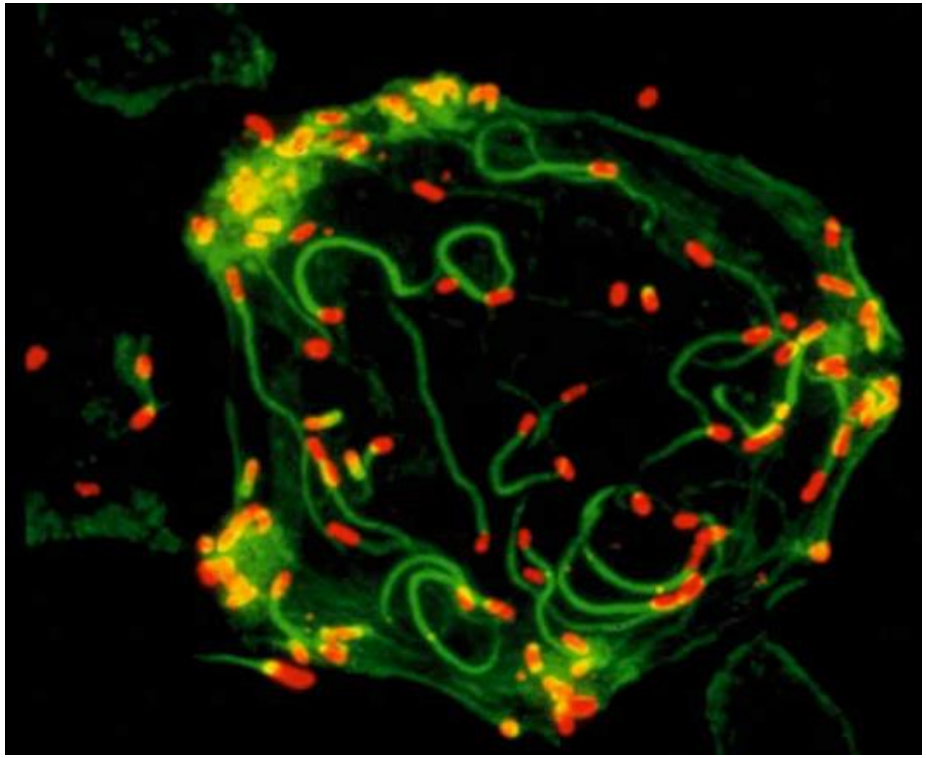
Loss of OmpT protease

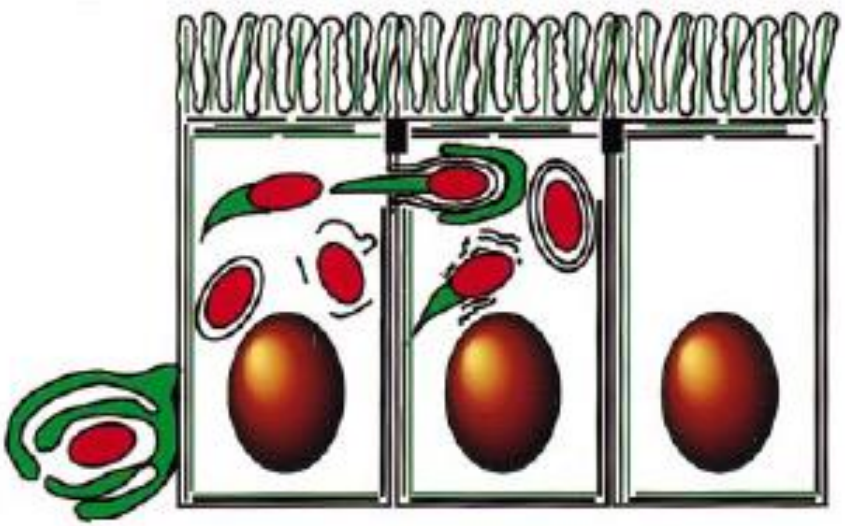
All *Shigella* and EIEC strains have lost the OmpT encoding gene

Motility of *Shigella* is mediated by plasmid-encoded protein IcsA

pINV

Shigella is able to infect epithelial cells, and to move intra- and inter-cellularly, using an actin-mediated motility

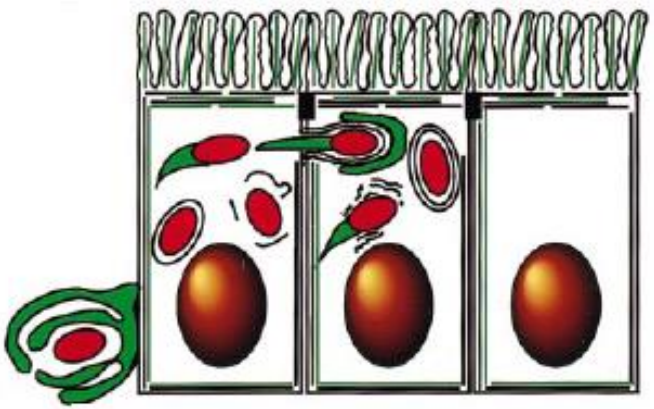


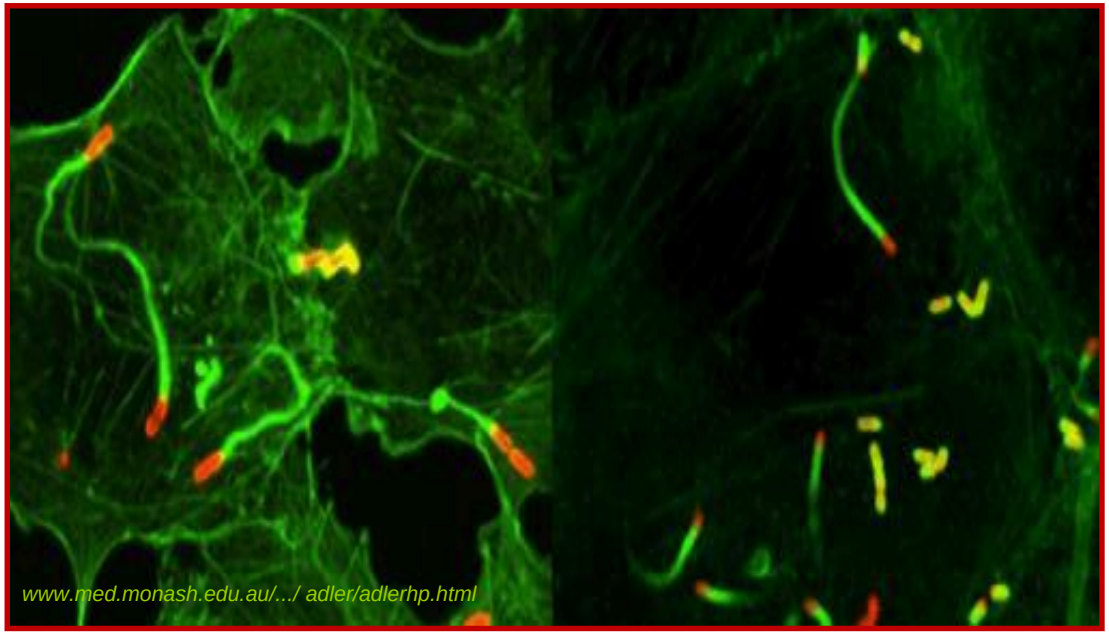


IcsA induces a rearrangement of the host cytoskeleton by assembling actin tails at one pole of bacterium

The loss of the OmpT protease is a pathoadaptive mutation in *Shigella*

The absence of OmpT, a surface protease, is an essential requirement for the ability of *Shigella* to spread intra- and inter-cellularly





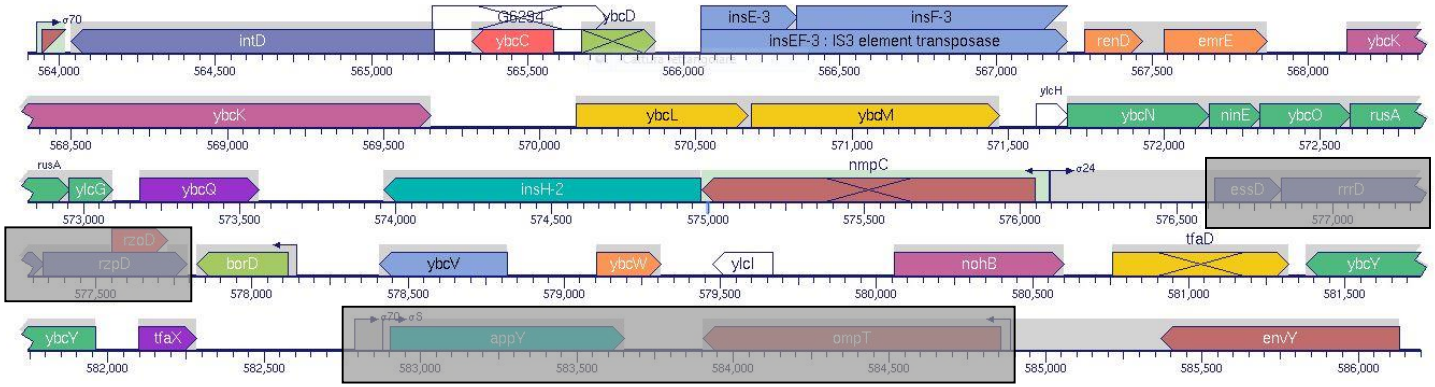
OmpT degrades the *Shigella* IcsA protein which is responsible for the formation of the actin tails at one pole of the bacterial cell

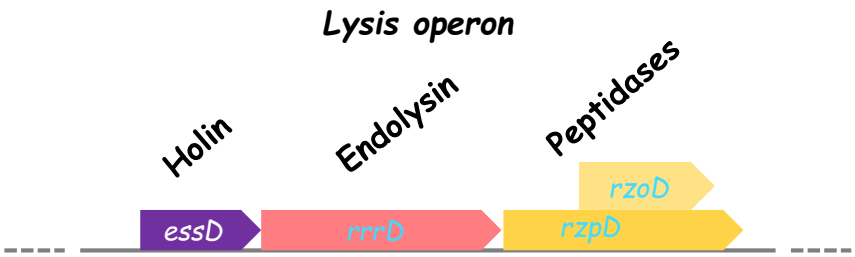
Il sistema lisina endolisina dei fagi

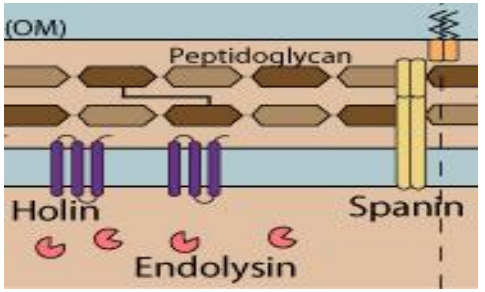
Il gene S codifica per olina, una proteina che crea dei fori nella membrana e per il suo inibitore trascrizionale che ne diminuisce la concentrazione, il gene R codifica per l'endolisina l'enzima che degrada i legami glicosidici del peptidoglicano mentre RZ e RZ1 codificano per proteine coinvolte nella rottura dei legami peptidici del peptidoglicano

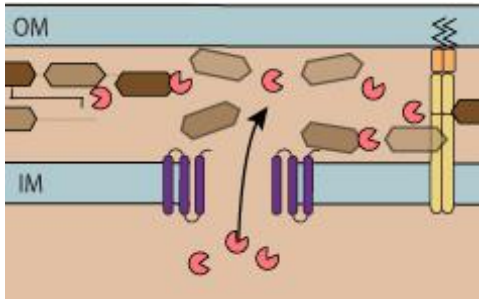
La cassetta LC è conservata nei fagi lisogenici di *E. coli* DLP12, Sp5, Sp6 e ha elevata omologia con la cassetta di Lisi dei fagi P21 e P1.
La cassetta LC di DPL12 è stata addomesticata da E.coli e utilizzata nel mantenimento della parete cellulare e nella formazione dei biofilm.

……not only *ompT* but also the DLP12 genes encoding the Holin/ Endolysin system are lost in *Shigella*

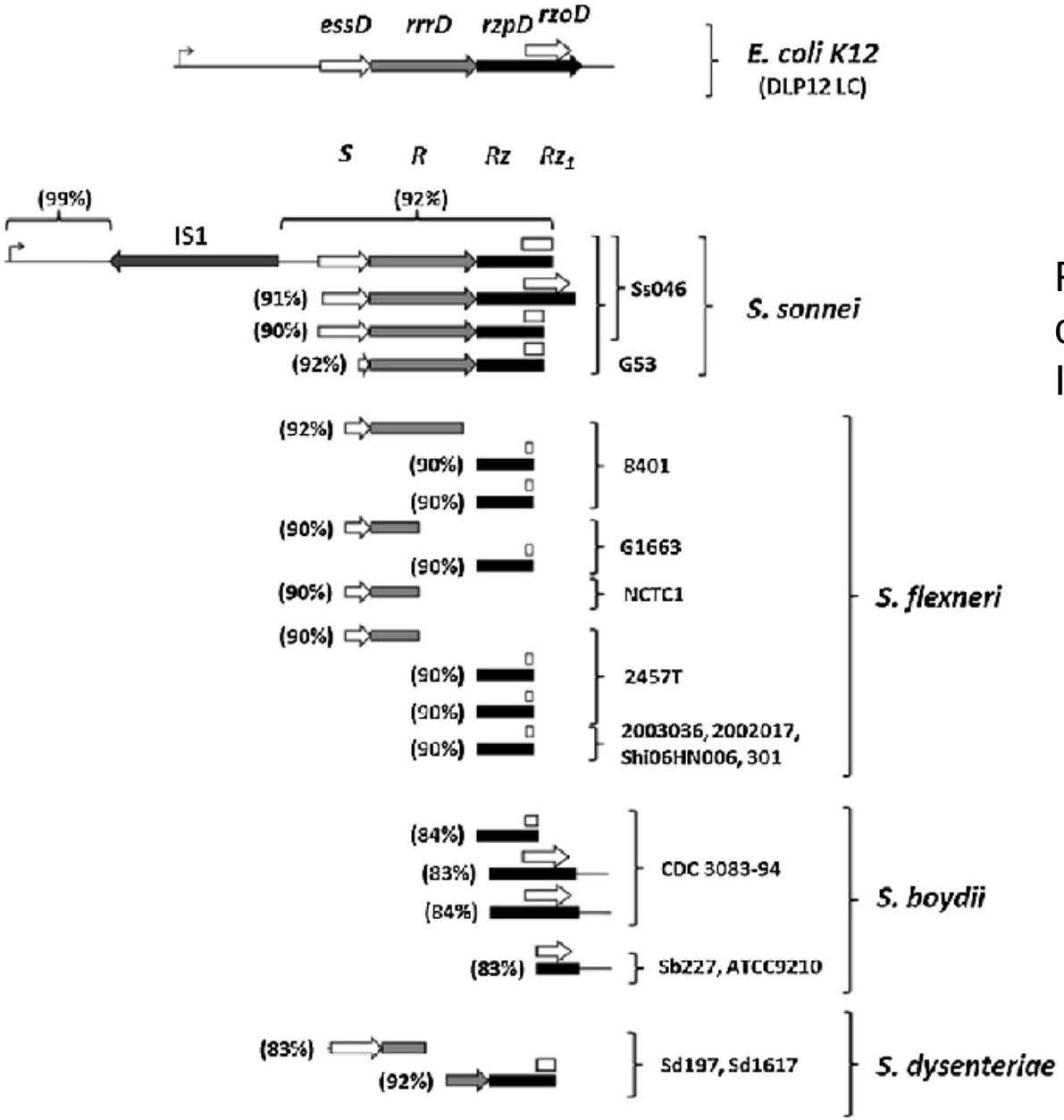








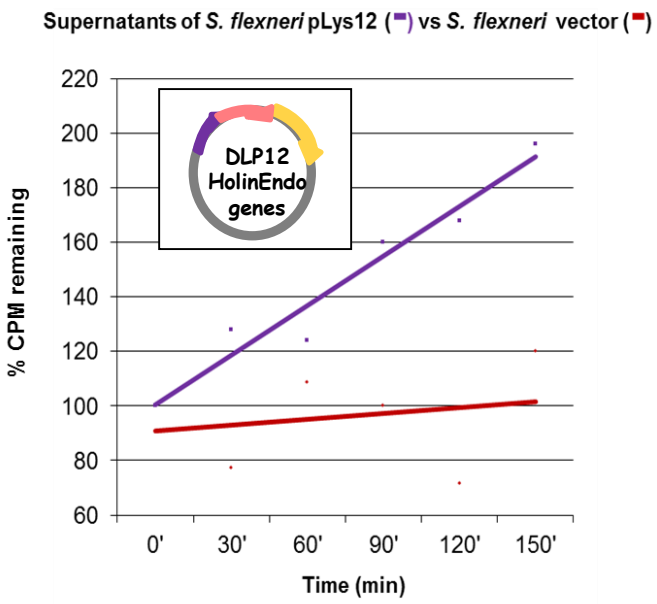
The Holin / Endolysin system of DLP12 has been "adopted" by *E.coli* and appears to be involved in remodelling of peptidoglycan during cell division and in the release of not recyclable PNG fragments

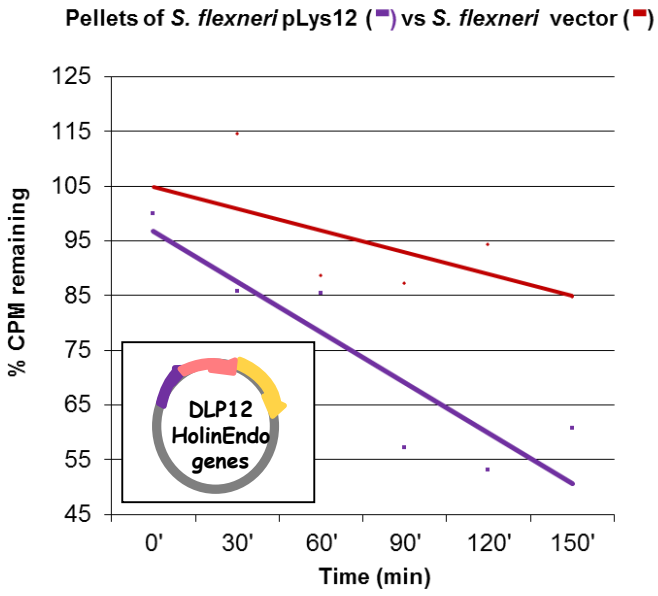


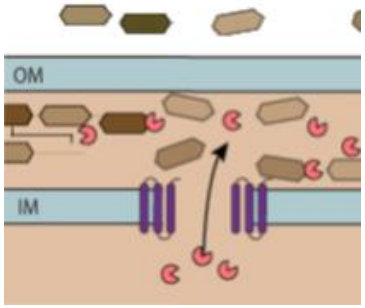
Riarrangiamenti nella cassetta di lisi DLP 12 In *Shigella*

Does the introduction of Holin/Endolysin of DLP12 prophage into *S. flexneri* increase the release of peptidoglycan components ?

[6-^{3}H] Glucosamine Peptidoglycan assay

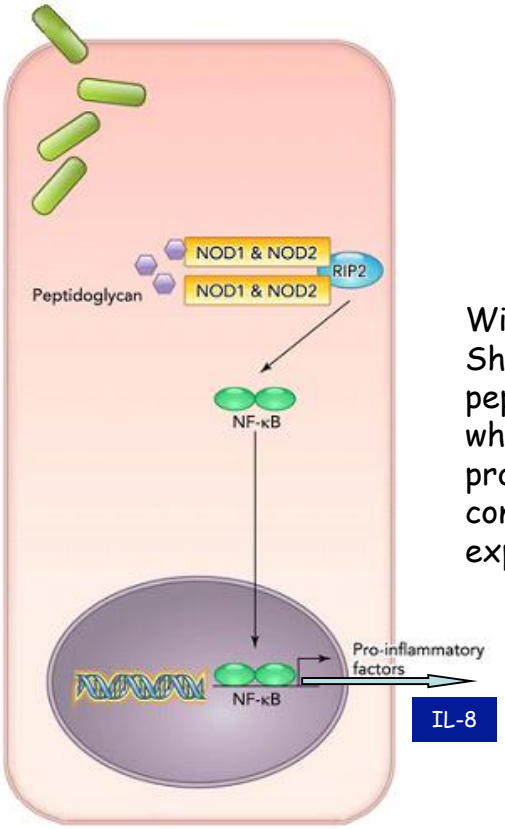




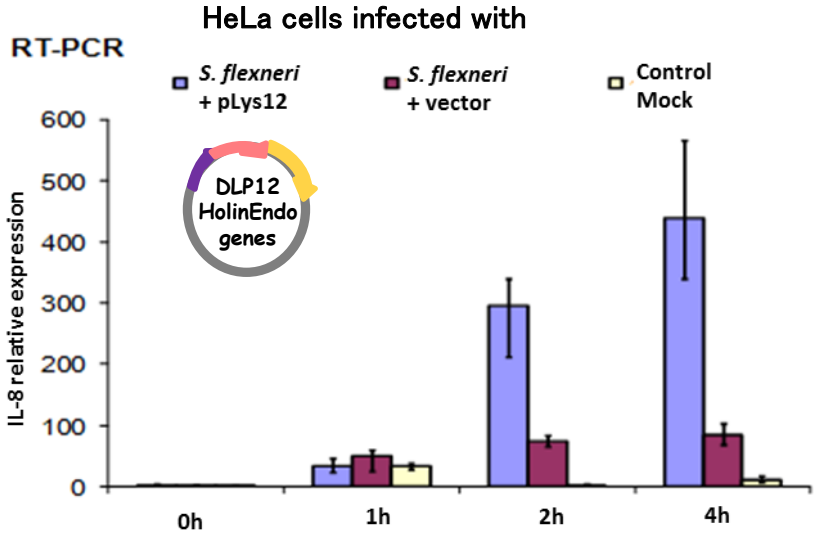


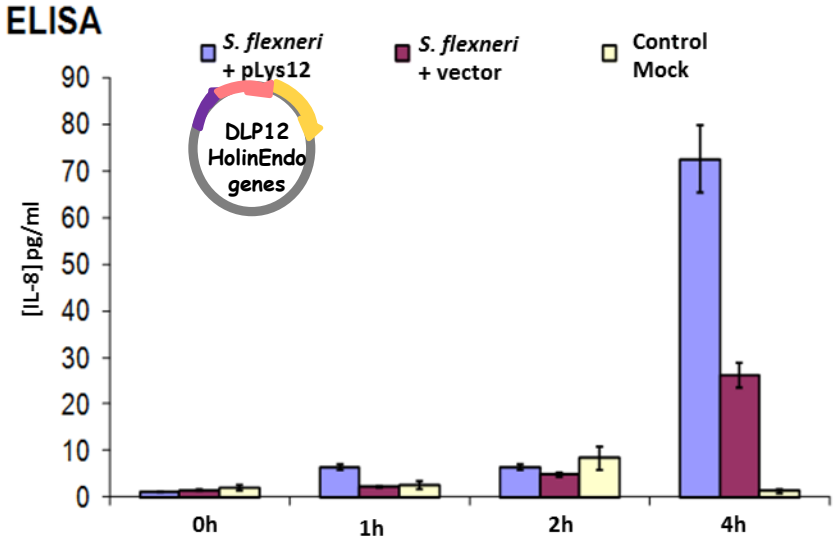
There is a strong increase of labelled peptidoglycan fragments in the supernatant of *Shigella* strains expressing the Holin/Endolysin system

Is the loss of the Holin / Endolysin System of DLP12 prophage a strategy to reduce the inflammatory response of the host?



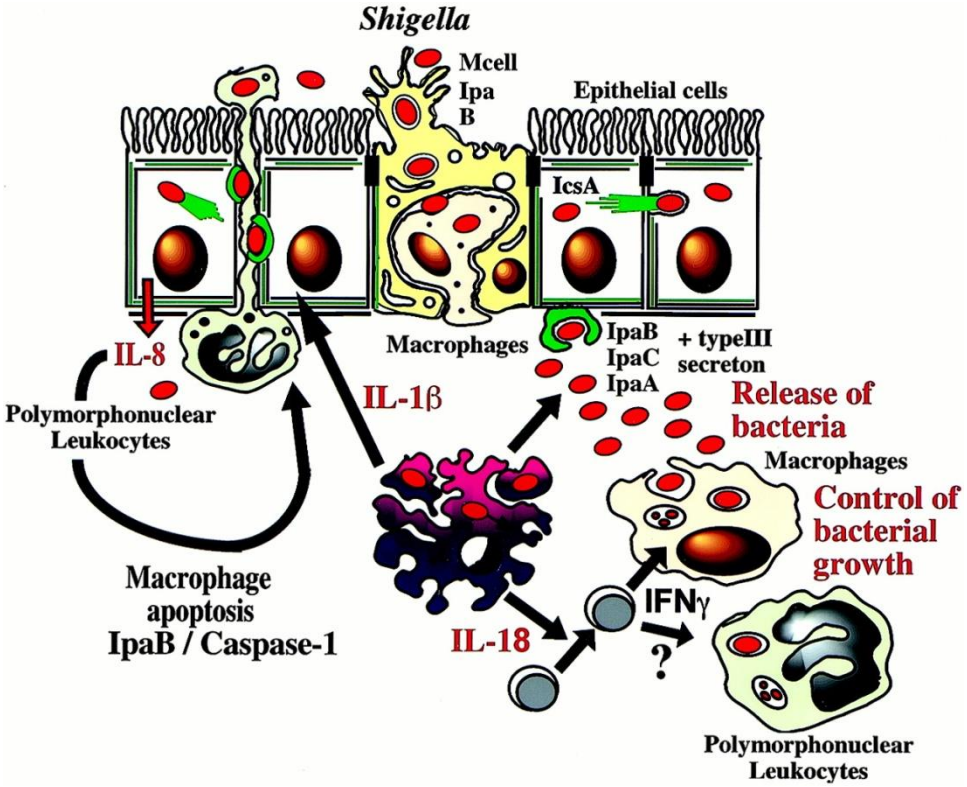
Within epithelial cells Shigella releases peptidoglycan fragments which activate Nod1/2 proteins and, as a consequence, induce IL-8 expression





The introduction of the pLys12 plasmid, carrying the Holin / Endolysin system of DPL12, leads to a strong increase of IL-8 and to the subsequent stimulation of the host immune response.

Shigella is able to induce an inflammatory response and to exploit it to optimize the invasive process.



The lack of the "lysis box "of phage DLP12 may be regarded as a new patho-adaptive mutation, necessary to avoid the massive inflammatory host response which would lead to the elimination of *Shigella*.

The long path of *Shigella* towards pathogenicity may involve more pathoadaptive mutations……