pathogens

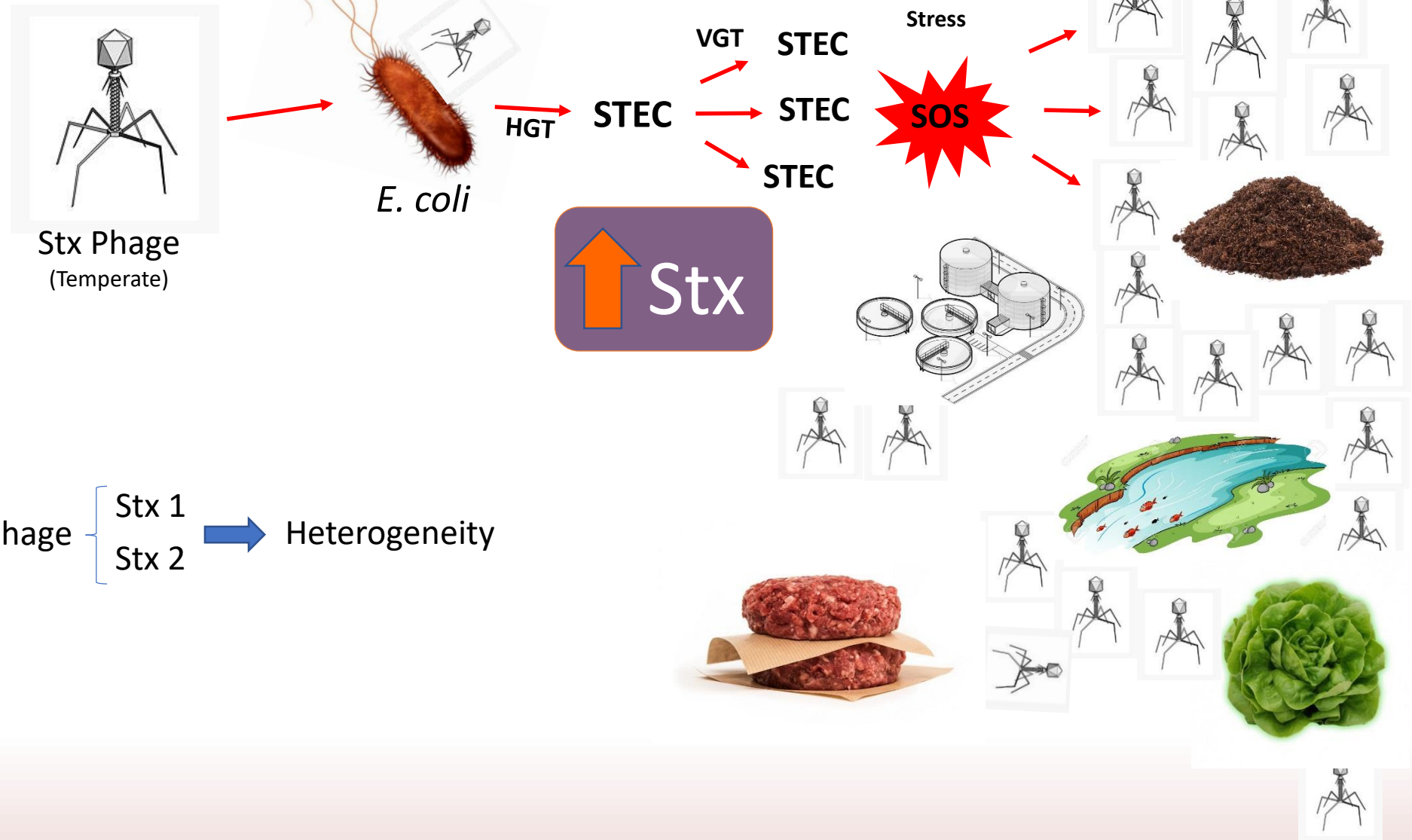
2021, 10:404

Bacteriophages of Shiga toxin-producing *Escherichia coli* and their contribution to pathogenicity

Lorena Rodríguez-Rubio, Nadja Haarmann, Maike Schwidder, **Maite Muniesa**, Herbert Schmidt

[1] Department of Genetics, Microbiology and Statistics, University of Barcelona
[2] Department of Food Microbiology and Hygiene, Institute of Food Science and Biotechnology, University of Hohenheim

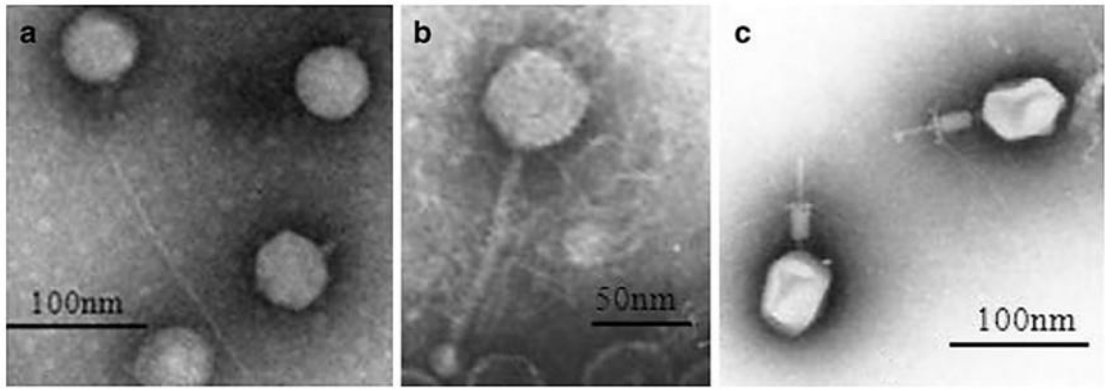
Stx phages

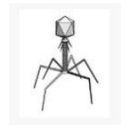


Phage { Stx 1 / Stx 2 } → Heterogeneity

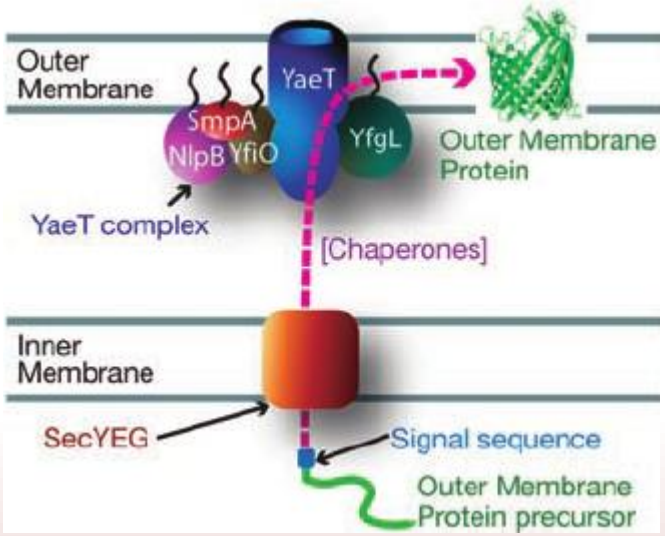
Stx phages

(42.6 – 66.4 Kb)





use highly conserved tail spike proteins for host recognition

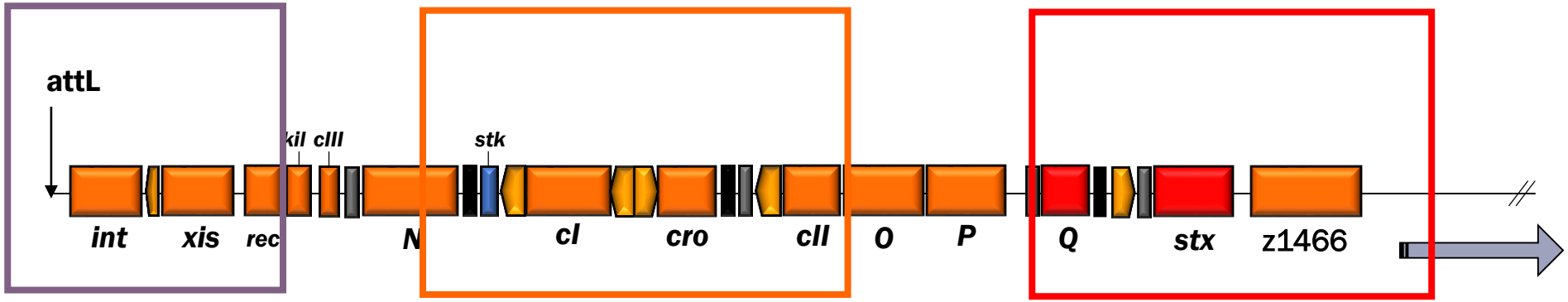


highly conserved receptor protein **YaeT** (BamA) on the bacterial cell surface and its orthologues

→ Spread among *Enterobacteriaceae*

Involved in protein colonization of OM and cell-to-cell contact inhibition

Stx phage genome



Integration/
excision

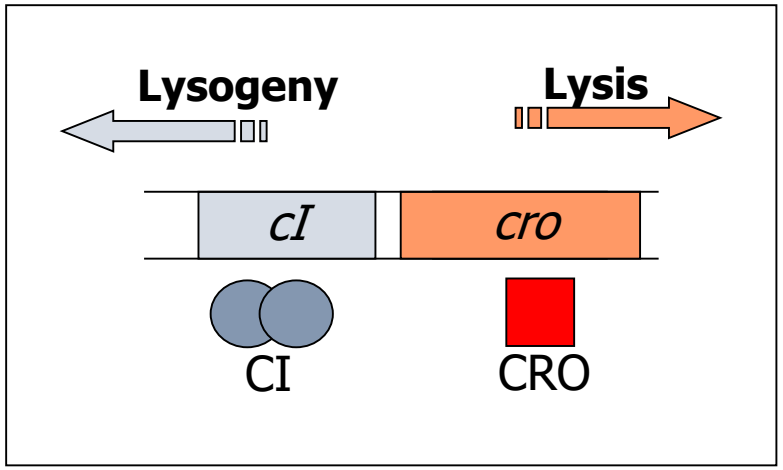
Lysis/lysogeny

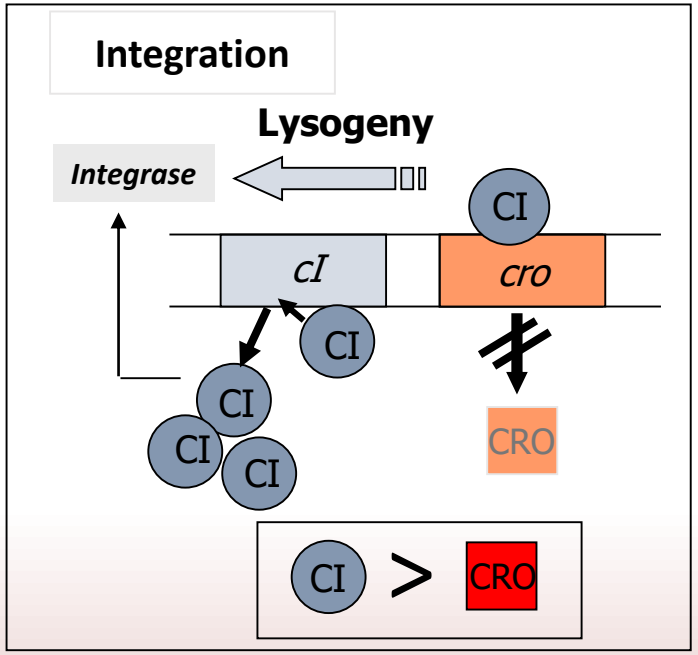
Lysis/Stx

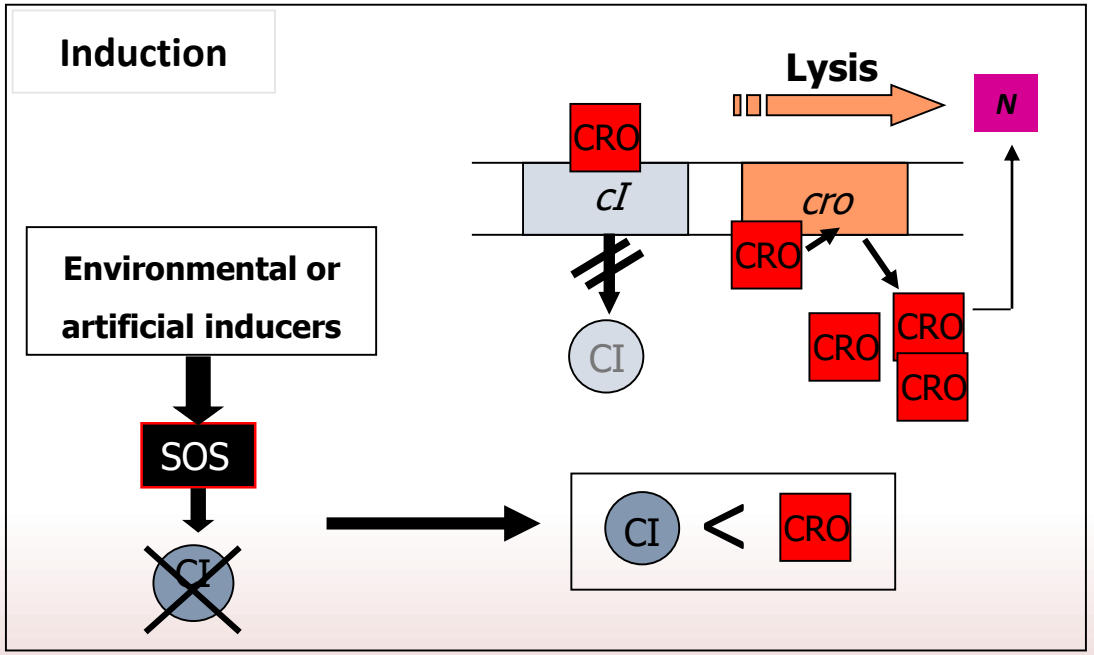
Structural genes
-Capsid
-Tail

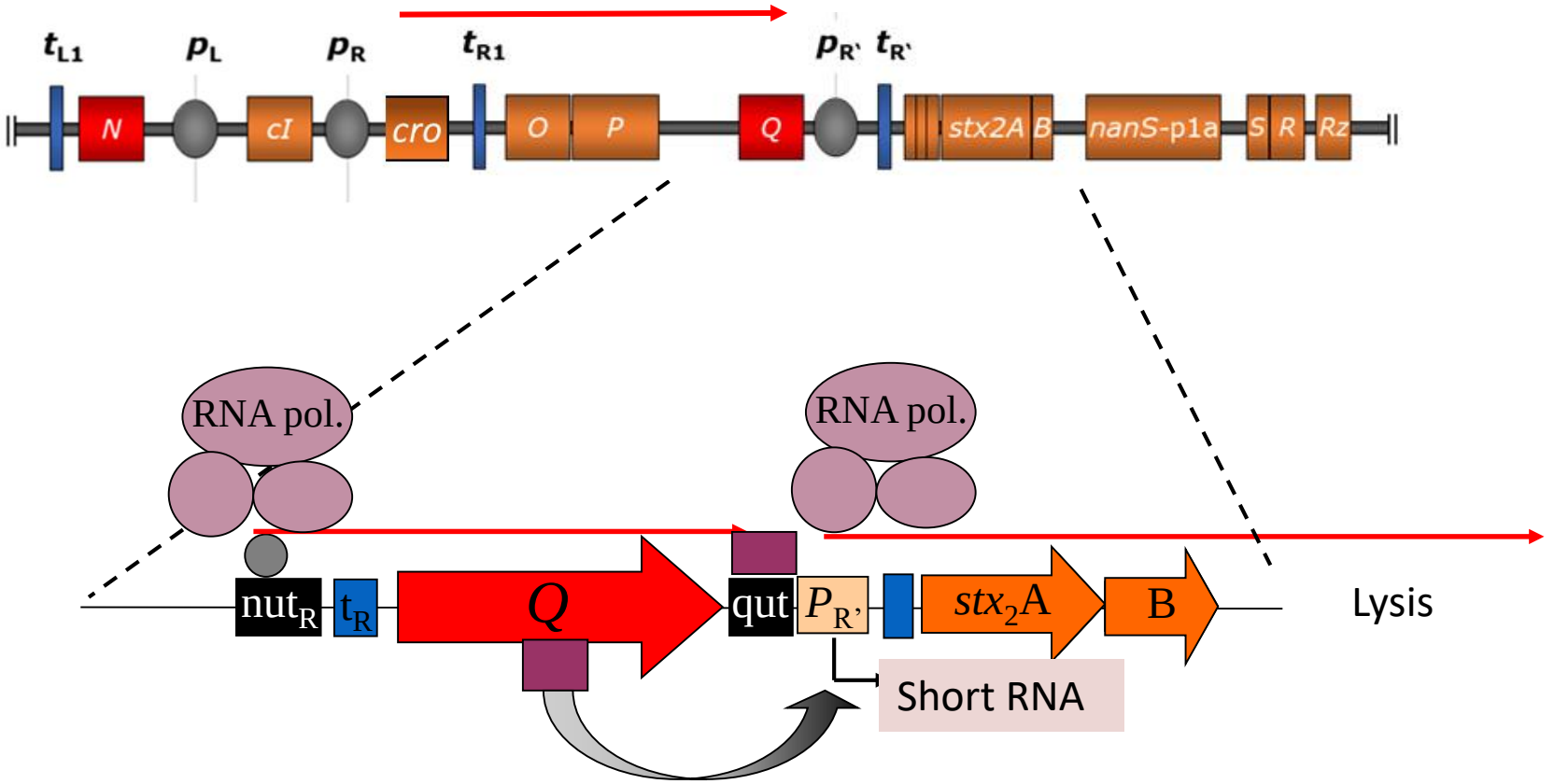
Stx phage induction

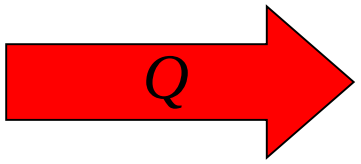
Model lambda lysis-lysogeny







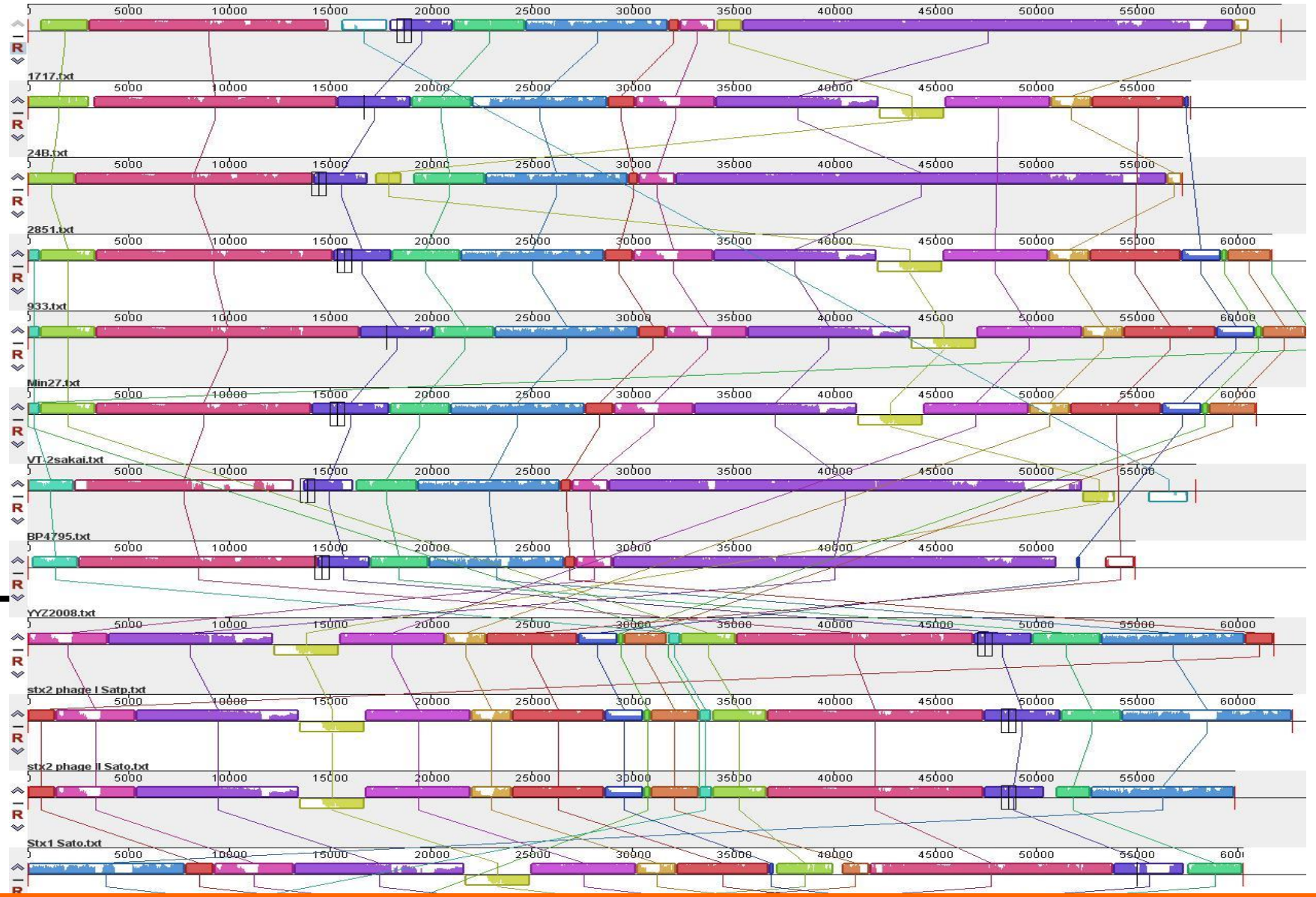




Some Q variants might be correlated with different Stx expression (Q_{21} lower, Q_{933} higher)

Differences in the number of operator regions, higher spontaneous induction

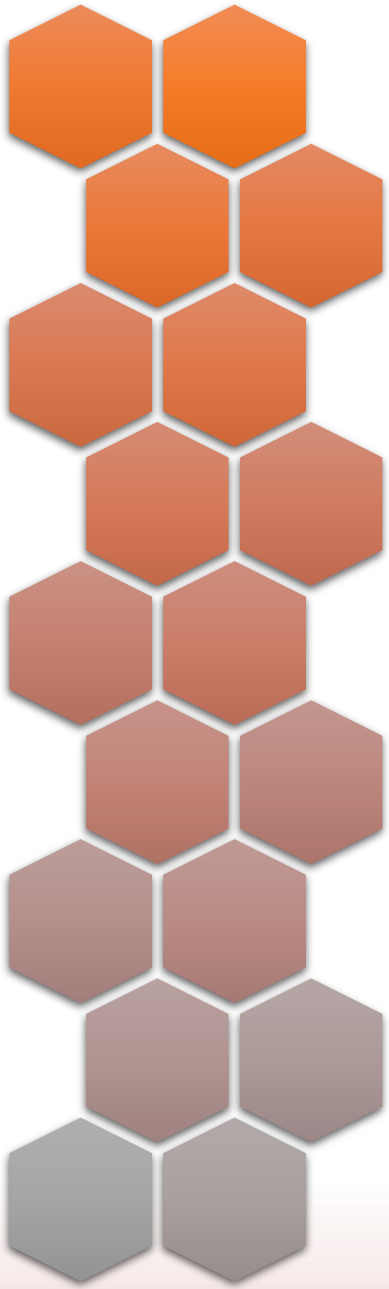
Stx2 phages



Stx1 phages

Polylysogeny: Genetic exchange between phages in the same STEC genome

Phage evolution: There are not two phages identical after passing through a new host

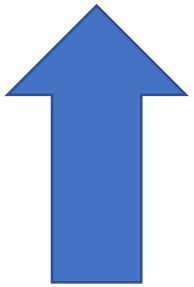


Stx phages Chromosomal insertion sites

yehV: curli expression	*TorS-torT*
wrbA: Trp repressor-binding protein	*potC*
yecE: ?	*uciD*
sbcB: exonuclease	*unfH*
Z2577: oxidoreductase	*serU*
ssrA: mRNA	*yjbM*
prfC: Peptide chain release factor	*yjjG* (Stx2h phages)
argW: tRNA	*dusA, yccA, zur* (Stx2k phages)

Promiscuous integrase

No phage immunity for Stx phages!! Superinfection with the same phage



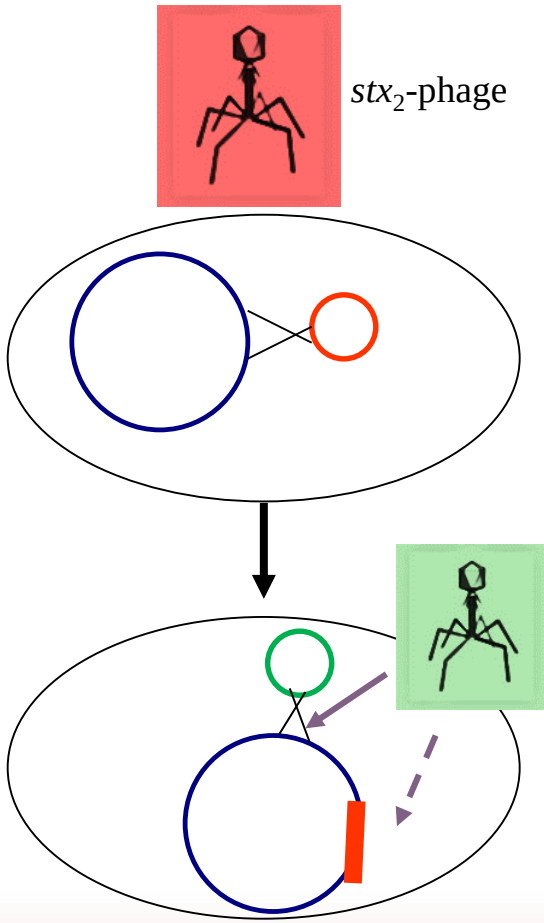
Toxin production??

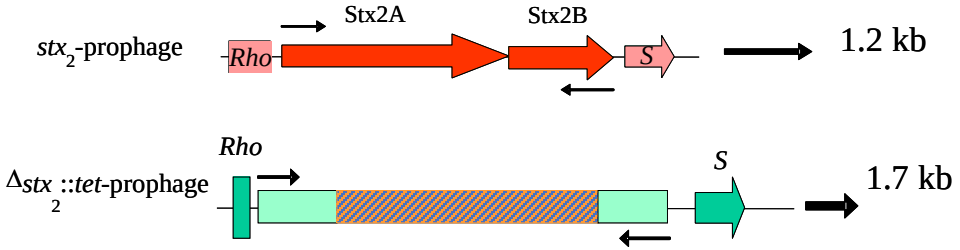
Double and even triple lysogens of Stx phages

Double and even triple lysogens of the same Stx phage can be generated

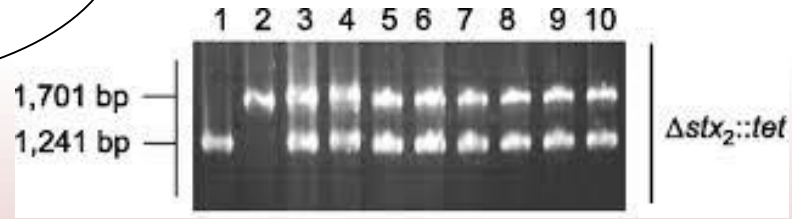
Stx phages (integrase) have a preferred insertion site. If it is not available (truncated or mutated, they search for a **secondary site for insertion**.

Promiscuous integrase





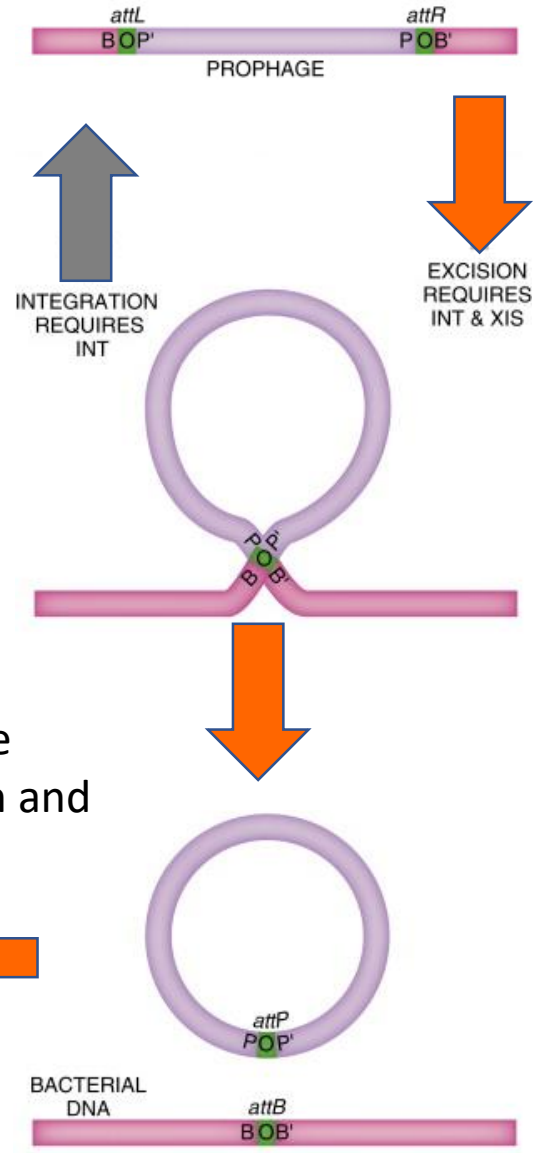
Double lysogen (stx_2-prophage + Δstx_2::*tet*/*cat*-phage)



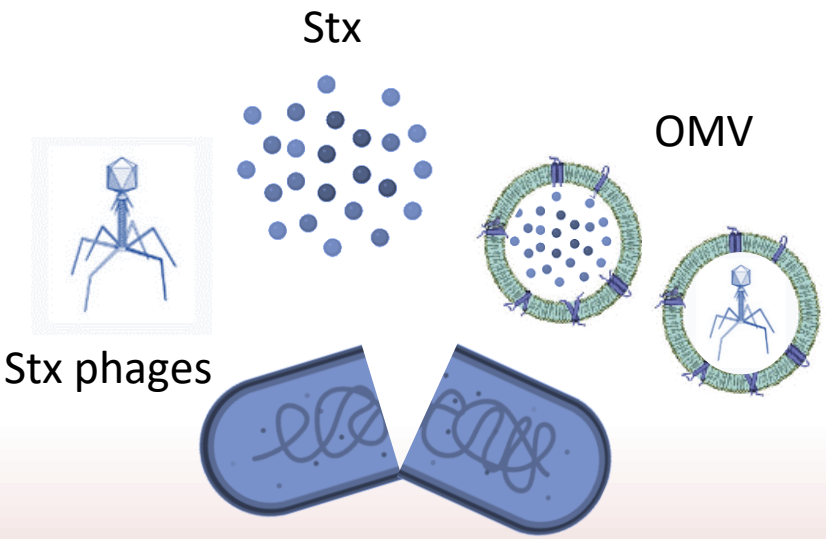
Induction, Expression and Release

Induction Factors

- UV
- MitomycinC
- Antibiotics
- Growth promoters
- H_2O_2
- pH changes (low pH)
- Ions (cations)
- Chelating agents (EDTA, sodium citrate)
- Fe depletion (Stx1 phages)
- ↓ AMPc



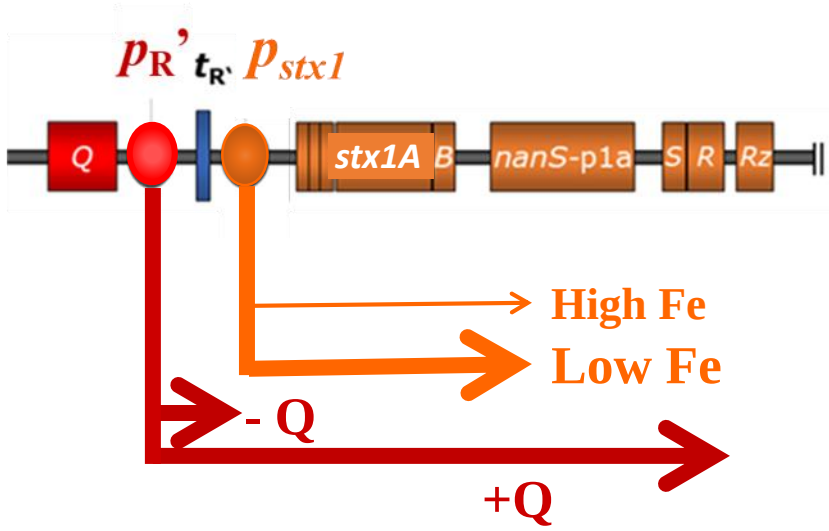
Phage formation and Lysis



Stx phages can be found as circular extrachromosomal element
(PFGE with S1 for plasmids)

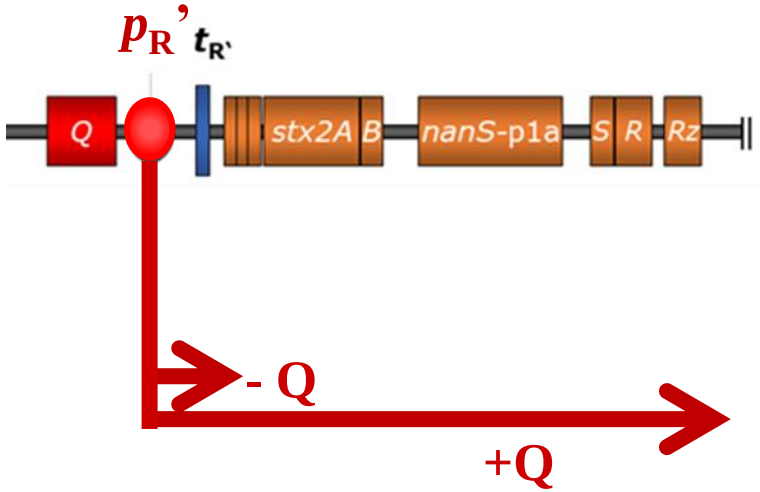
Differential Stx1 and Stx2 phage induction

Phages Stx1 and Stx2 are similar but display an important difference



Stx1 Phages

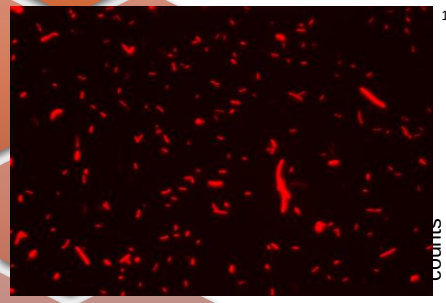
Stx1 can be expressed either by the induction of the phage or by a low concentration of iron in the medium

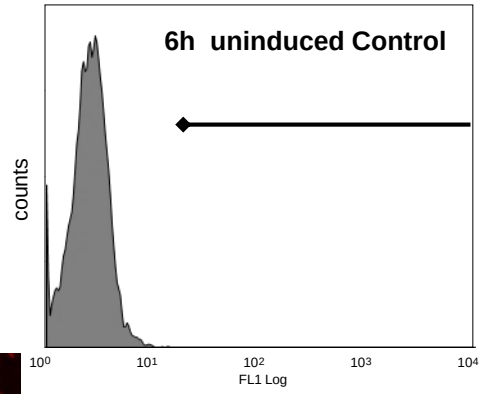


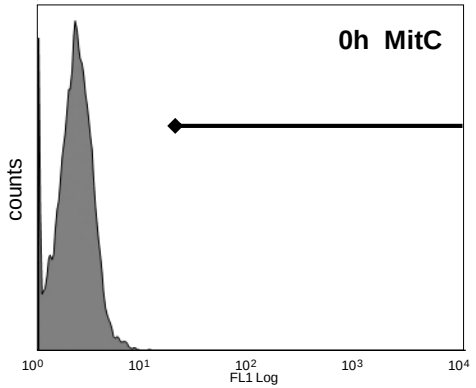
Stx2 Phages

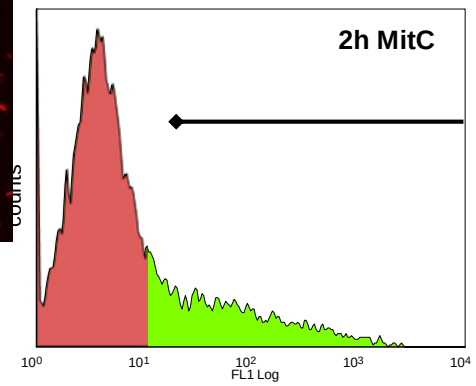
Stx2 can **only** be expressed by phage induction

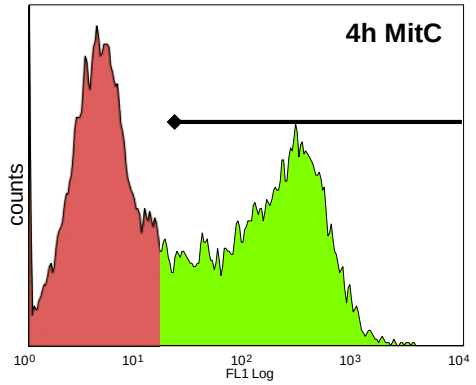
Differential Stx phage induction

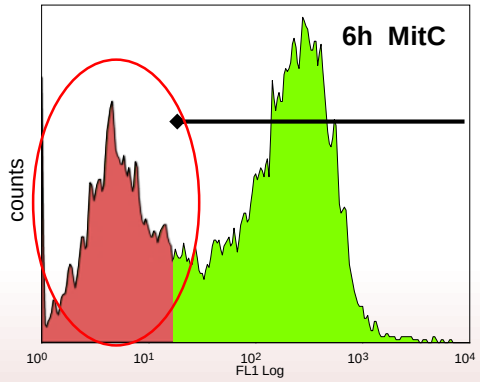


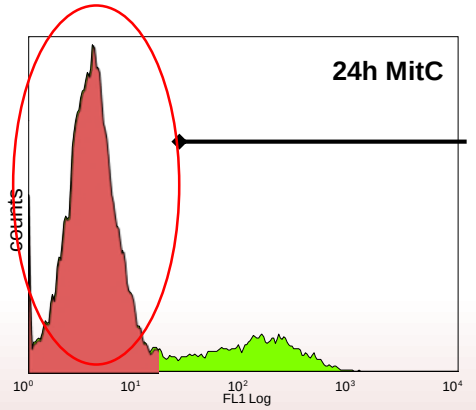








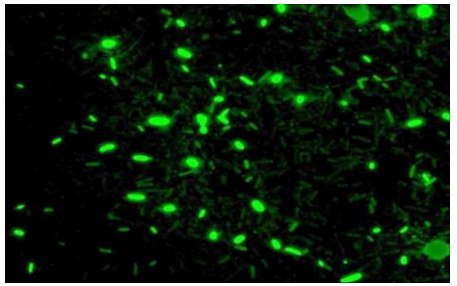


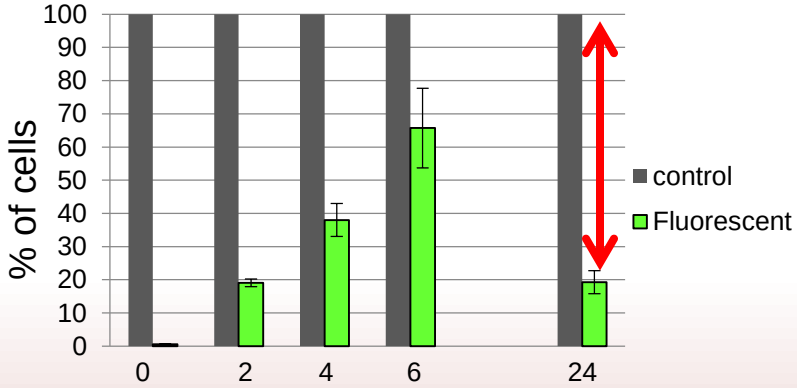


Two subpopulations could be distinguished after induction of the phages

One keeping lysogenic state

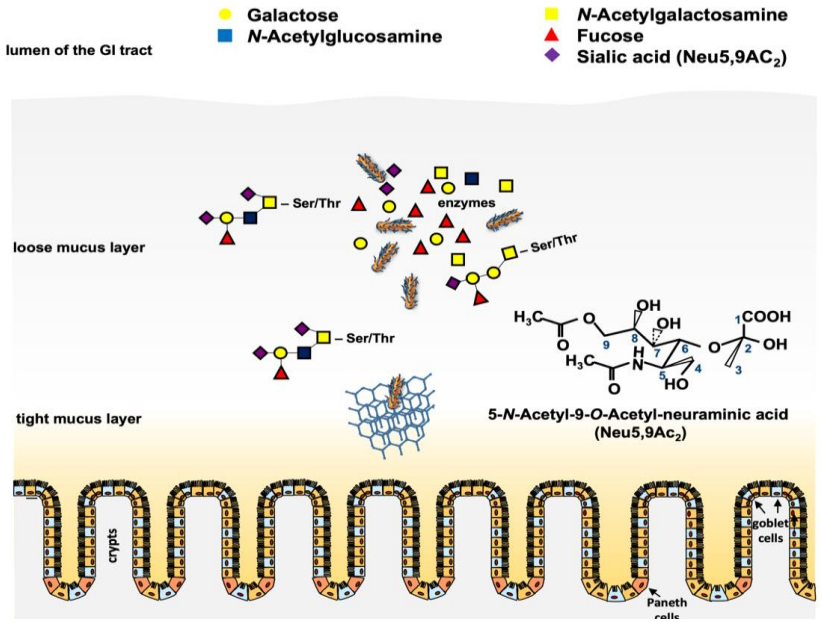
One inducing Stx phages





Other genes in Stx phages and regulation of STEC pathogenicity

Esterase function



Benefits for **STEC** that can use mucins as a substrate

or/and

Benefits the **Stx phage** that better recognize bacterial receptors or can use LPS as primary binding site (cleavege of O-acetyl group in O-antígens)

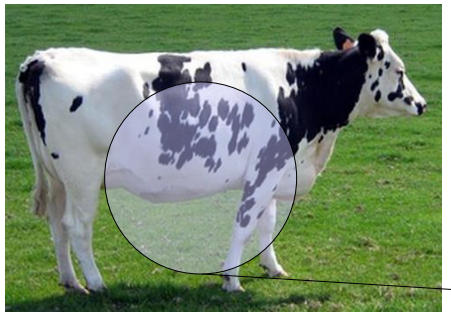
Still a lot to be said in the *exo-xis* region of the Sxt phage:

Excisionase-integrase

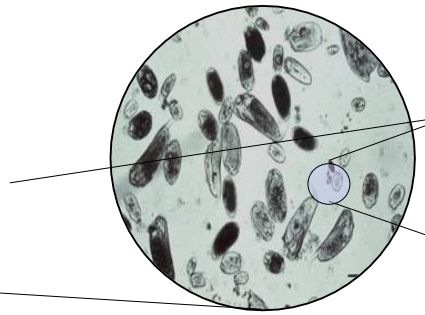
5 genes involved in induction *vs* maintenance of lysogeny

Evolucionary viewpoint: positive selection of STEC containing Stx phages

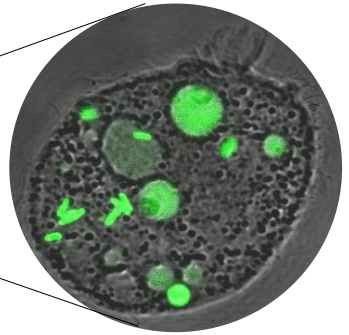
STEC reservoir



Rumen ciliate protozoa



Protozoa “eat” bacteria by fagocytosis



Stx may prevent grazing

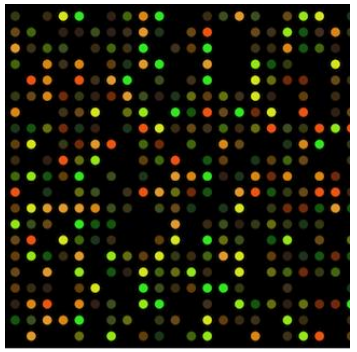
→ Killing protozoa → Good for caws

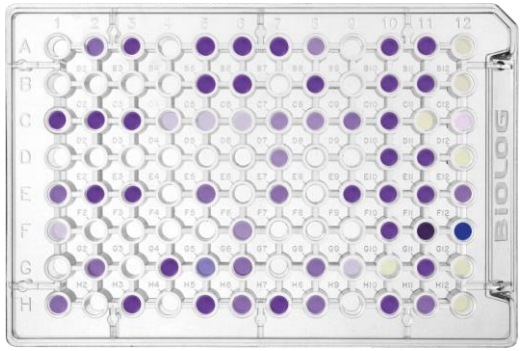
→ Increasing survival of *E. coli* in vacuoles

Some contradictory results...

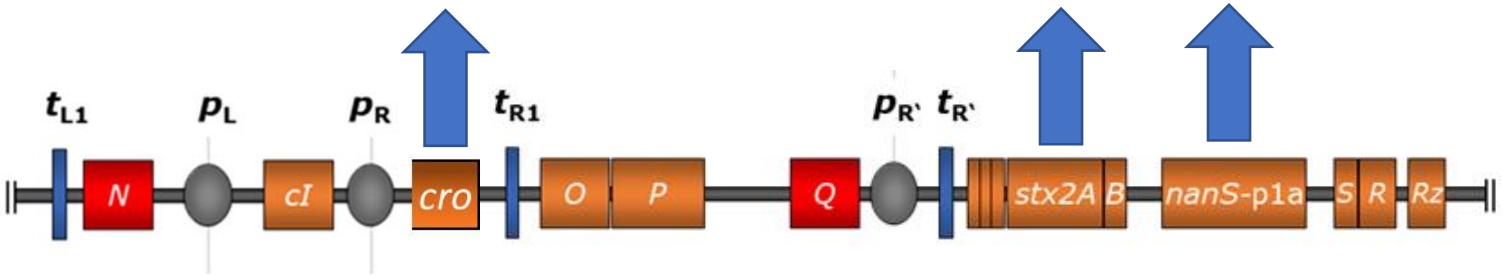
Steinberg and Levin, 2007; Lainhart et al., 2009

Influence of Stx phages in bacterial transcriptome





Late phage genes upregulation



Stx phage lysogeny

Better bacterial fitness in vivo ?

Depending on the host background (differences between STEC or lab strain)

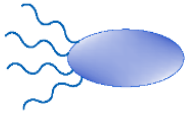
Influence of Stx phage in bacterial transcriptome → Presence of Stx phage or upon induction

Expression of GAD operon (glutamate-dependent acid resistant system)

Acid resistance

Anaerobic respiration

Motility and chemotaxis



fliC, *fliA* can be repressed or enhanced depending on conditions, (Cro?)
Sulfur motility enhanced

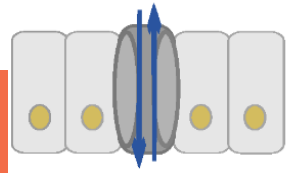
Anti Small-RNA in phage

"sponge" sRNA

sRNA

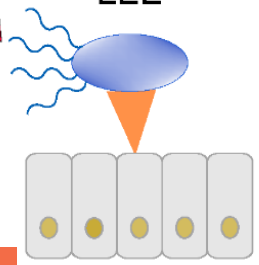
Small RNA in phage that regulate gene expression

Transport



C-, N-, energy & fatty acid metabolism

LEE



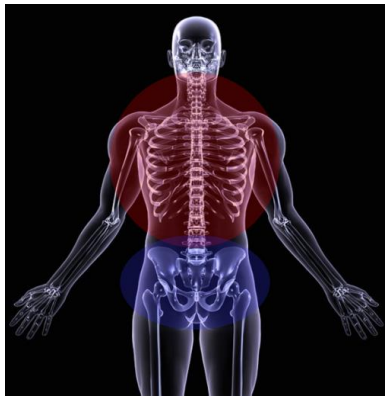
Reduced expression of ompC and ompF porins

Phage- suppressed genes of pyruvate decarboxylases (AcCo)

Xu et al. Regulation with LEE Stx phage causes suppression of T3SS

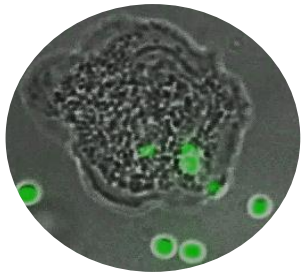
Related to Cro?
Regulators in *gam* anc *CII* gene?

Impact of Stx phages on human host

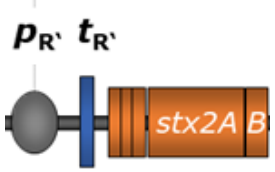


Stimulate phagocytosis

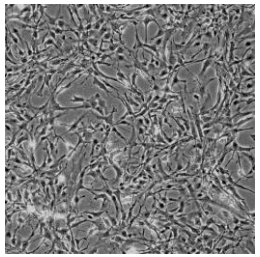
Generates Ab



www.evasionutrecht.nl



Stx sequence
(with promoter)



Stx production

C600(933W)

- Only Stx expression
- Transduction to other hosts
- Direct interaction phage-eukaryotic cell ??

Stx expression
(No phages)

- Modulates innate immune response, inhibits NF-kB an chemokines
- Favors attachment of STEC to the colonic epithelium (nucleolin)
- In animals, **Stx1** suppresses mucosa-associated immune response, **Stx2a** and **Stx2c** affects regeneration of epithelium in calves while **Stx2a** enhances O157 colonization.

New Stx phages

Stx1: a, c & d

Stx2:**a**, b, **c**, d, e, f, g

h: healthy marmots in China

i: shrimps and bivalves

j:??

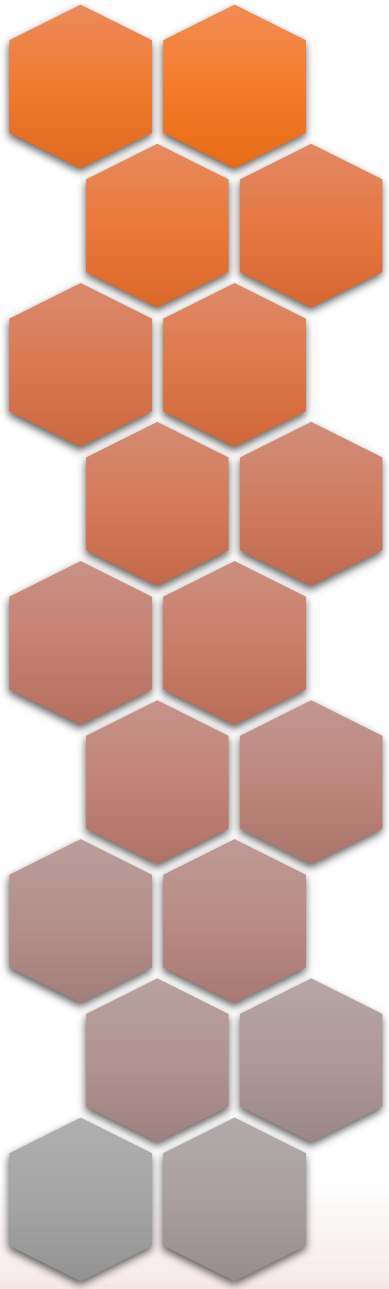
k:humans, animals and raw meat in China (possible hybrid STEC/ETEC pathotype with genetic heterogeneity

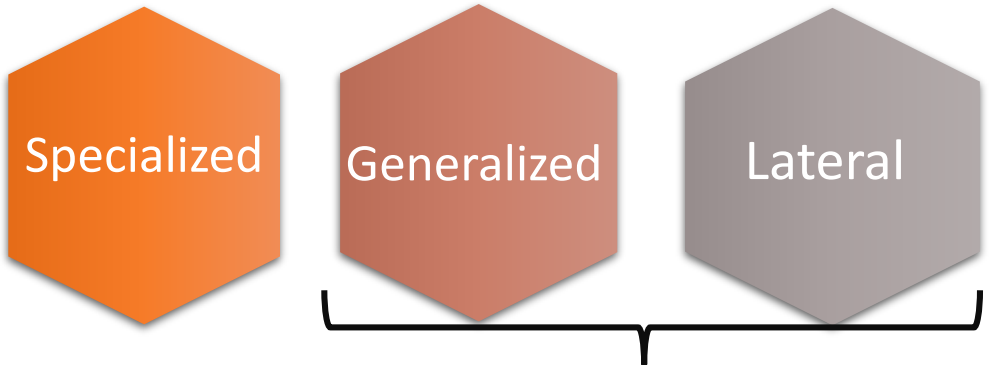
Variants and their level of expression are related with pathogenicity (**a / c**)

Other non-Stx phages in STEC

Type	Virulence factor
Toxins	Cdt
Adhesion	Lom
T3SS	Cif, EspF, ESpJ, EspL2, NleA, NleB, NleC, NleH, NleG, espJ, nleA/espI
Immunology	Immunoglobuling binding protein eibD,
Serum resistance	Bor
Metabolism	NanS-p
Antibiotic resistance	Betalactamases (bla), qnr, sul, tet, arm, str, etc

Horizontal gene transfer: transduction in Stx and non-Stx phages in STEC





Phage DNA in phage capsids + 1 fragment of DNA bacteria

Bacterial DNA in phage capsids

Genetic mobility

Stx phages mobilize plasmids with ARG

non-Stx phages also affects STEC

- Stress tolerance (pH, heat, osmotic oxidative)
- Increase biofilm formation
- Antibiotic resistance
- Protection against superinfection
- Changes in phenotype (curli generation)

Thanks!

Grazie!

Gràcies!

mmuniesa@ub.edu
Departament of Genetics Microbiology & Statistics
University of Barcelona

UNIVERSITAT DE BARCELONA