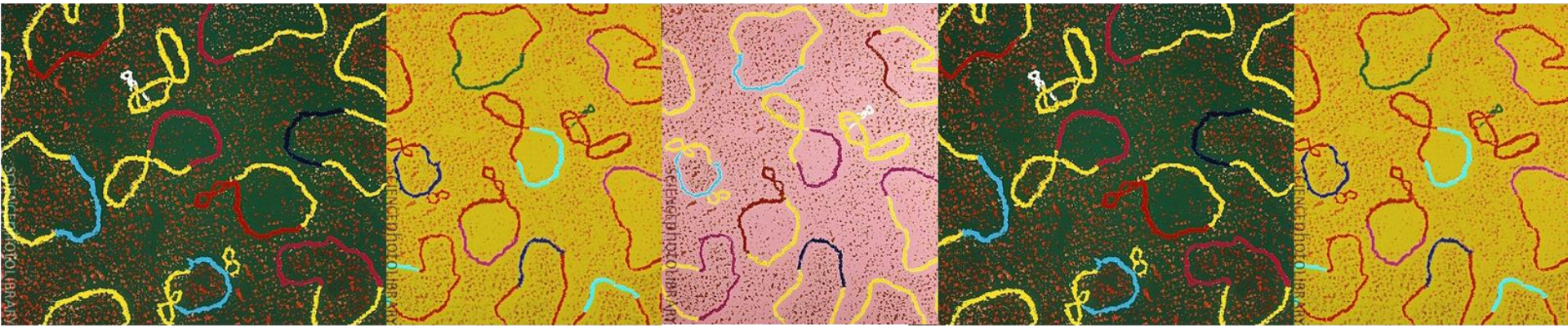
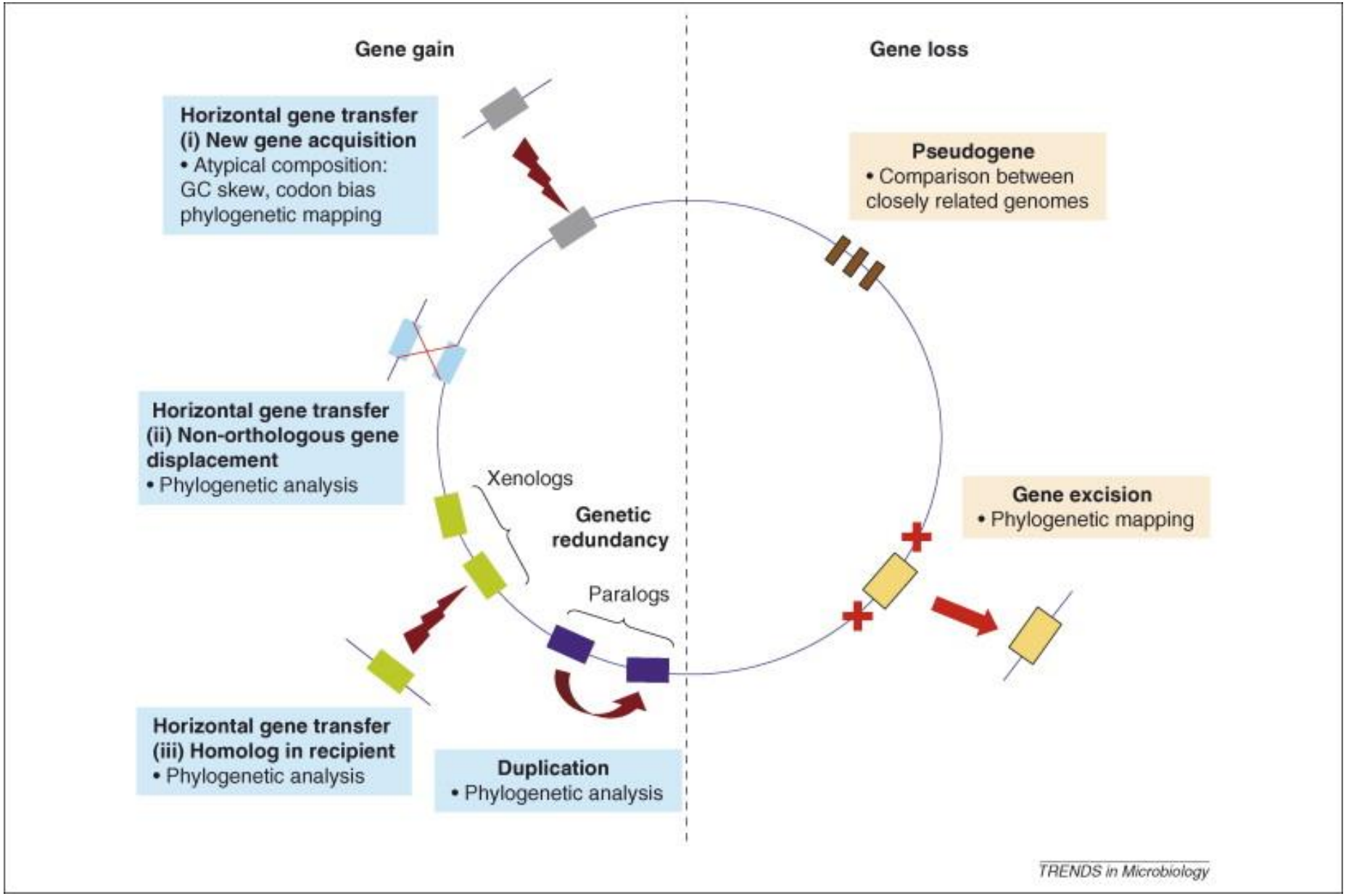
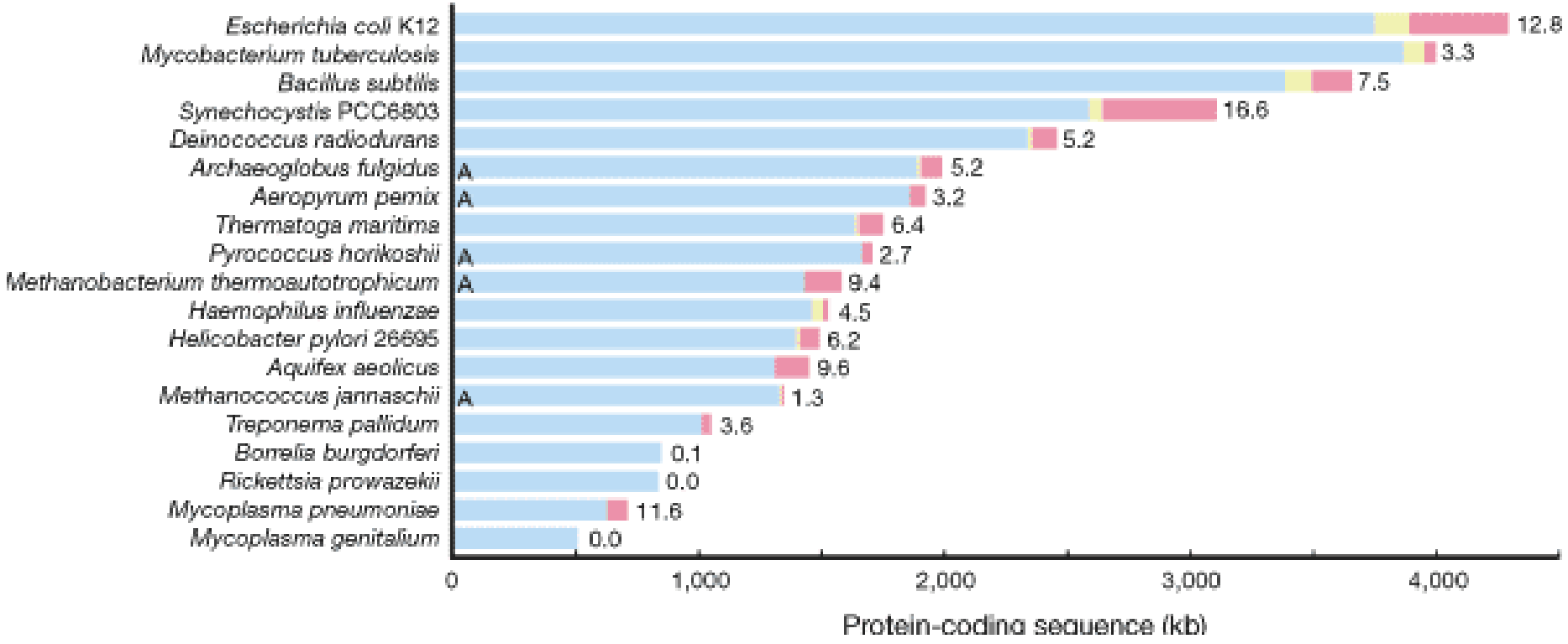
Horizontal gene transfer

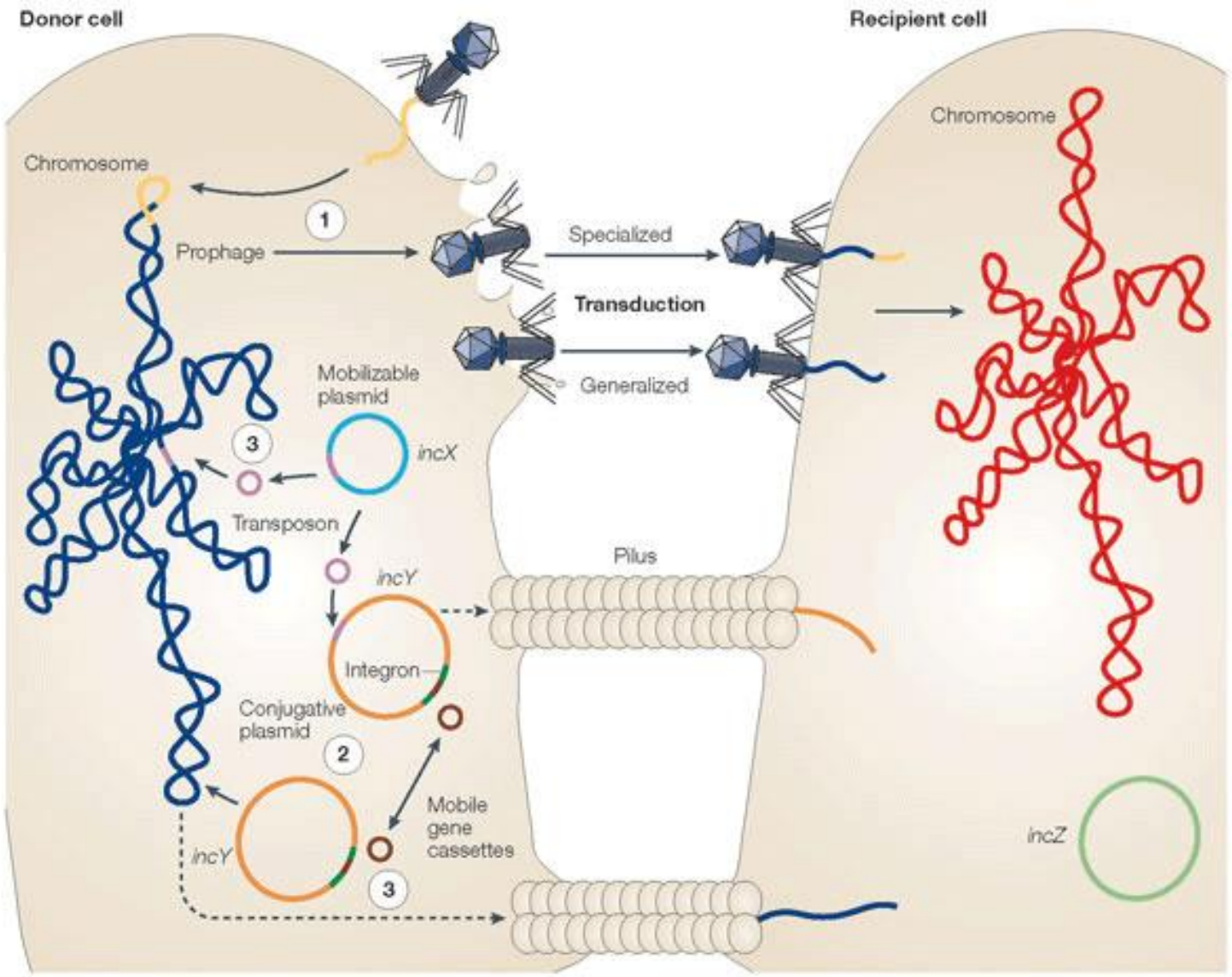



Gene gain
Gene loss
Horizontal gene transfer
(i) New gene acquisition
• Atypical composition:
GC skew, codon bias
phylogenetic mapping
Pseudogene
• Comparison between
closely related genomes
Horizontal gene transfer
(ii) Non-orthologous gene
displacement
• Phylogenetic analysis
Xenologs
Genetic
redundancy
Paralogs
Gene excision
• Phylogenetic mapping
Horizontal gene transfer
(iii) Homolog in recipient
• Phylogenetic analysis
Duplication
• Phylogenetic analysis
TRENDS in Microbiology

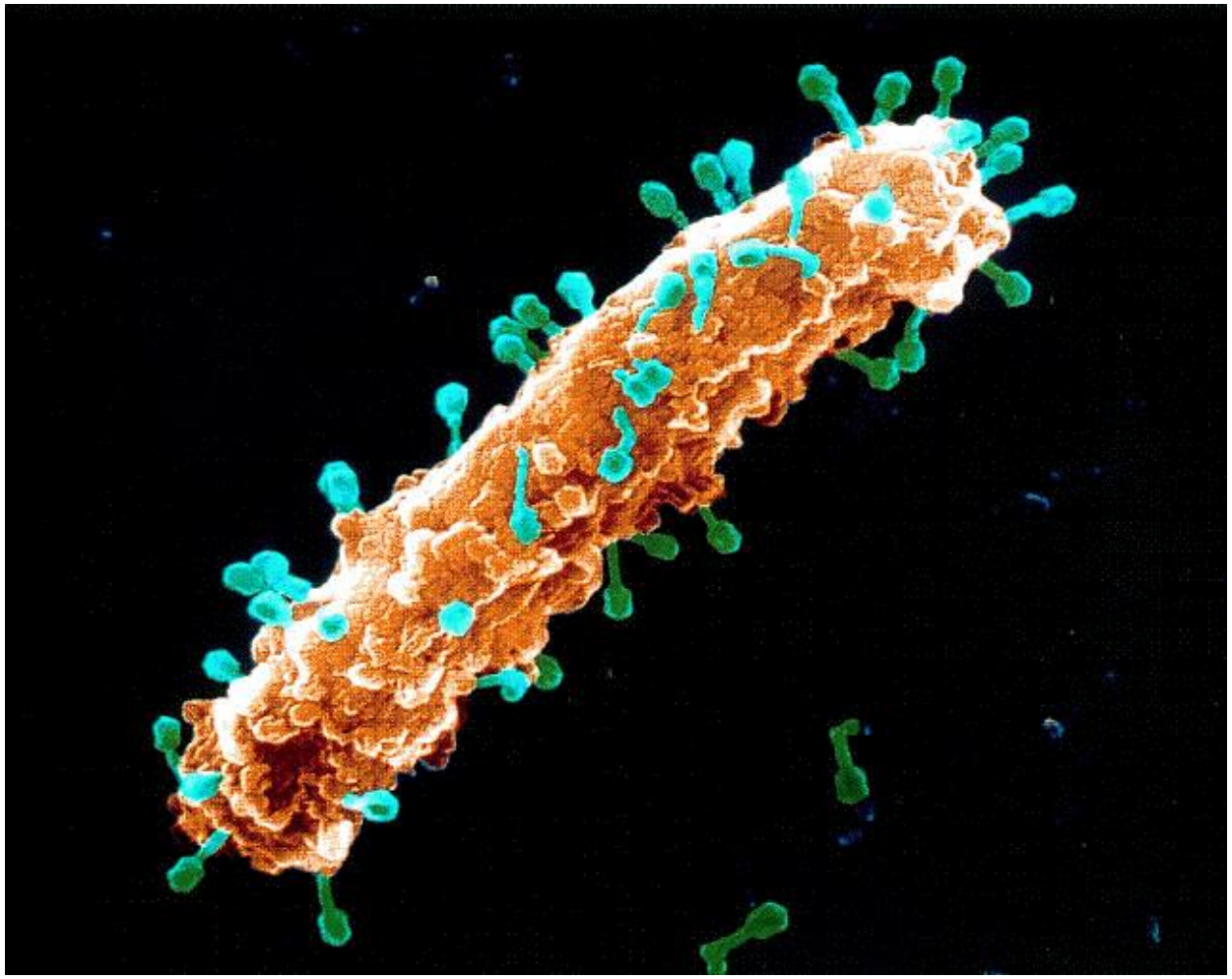


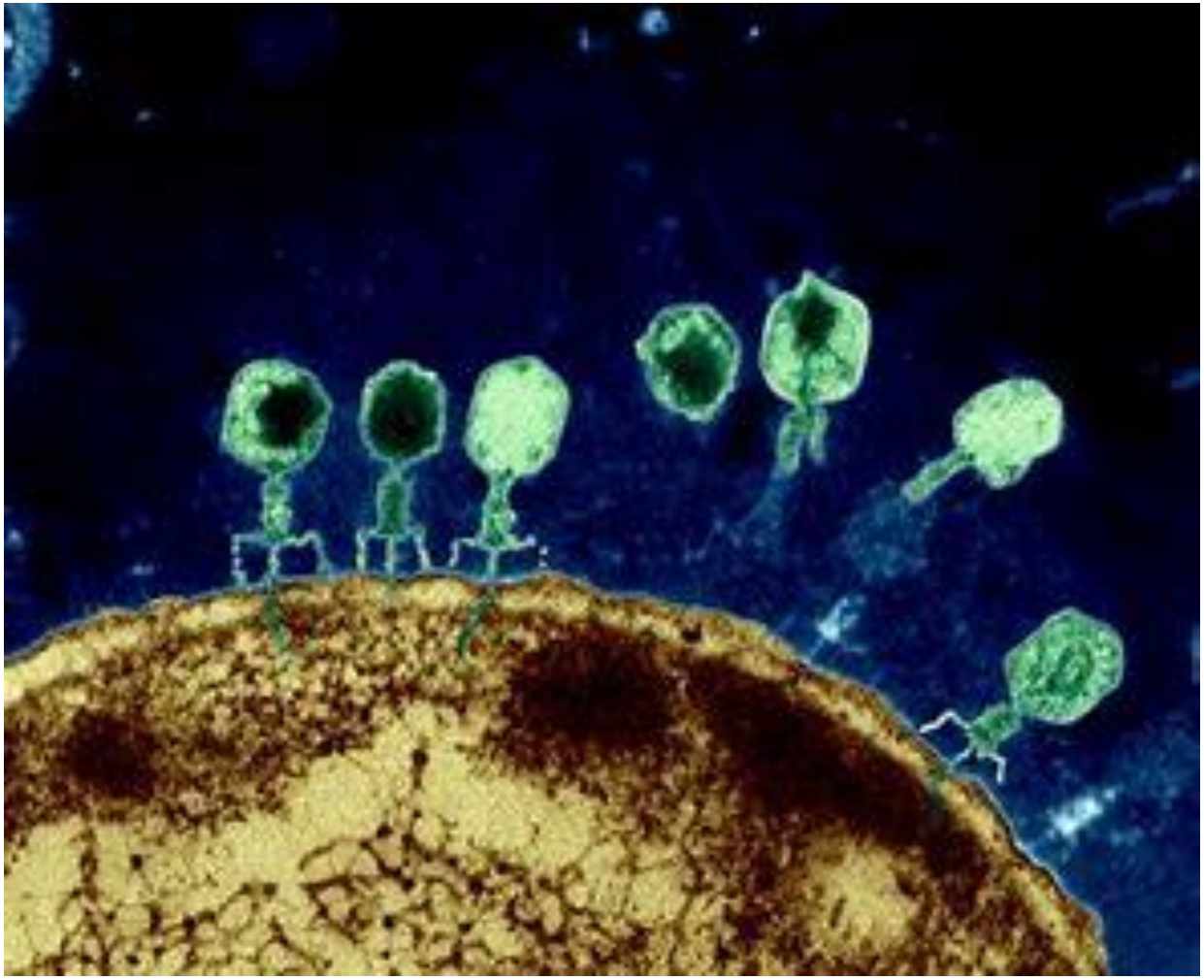
Native DNA in blue, Mobile elements in yellow and HGT DNA or alien DNA in pink

HGT mechanisms

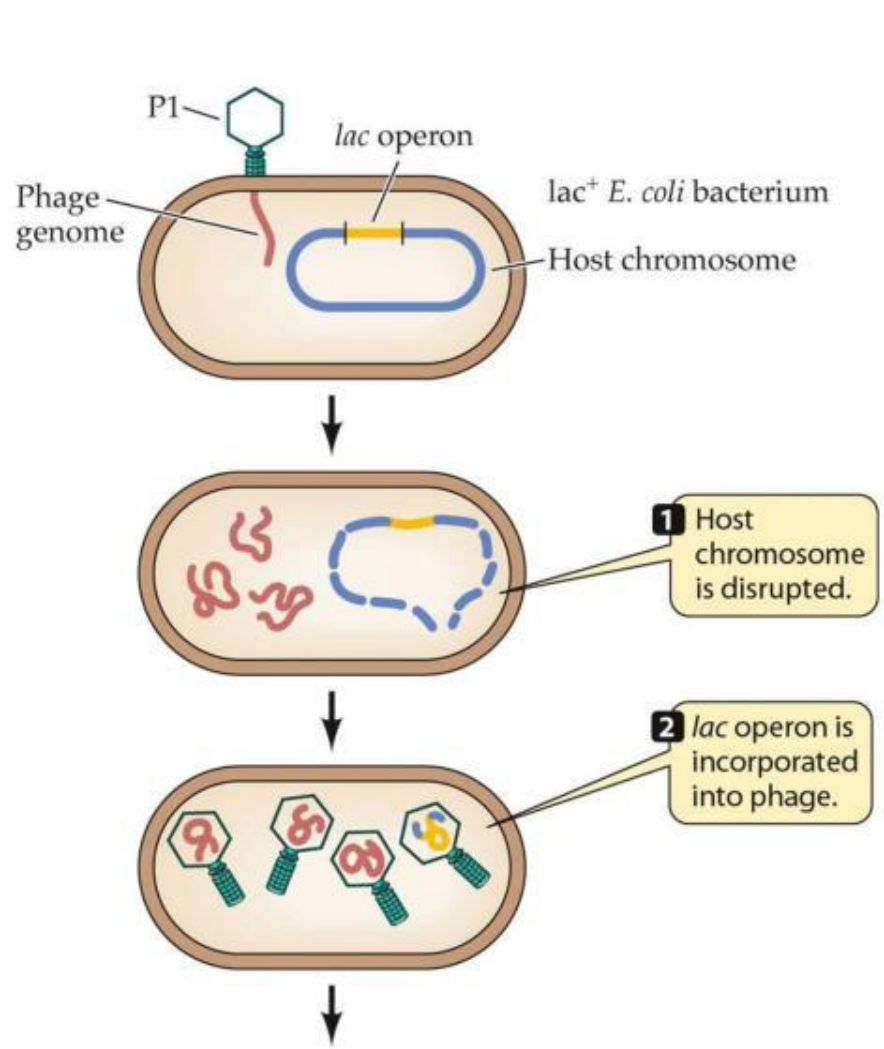


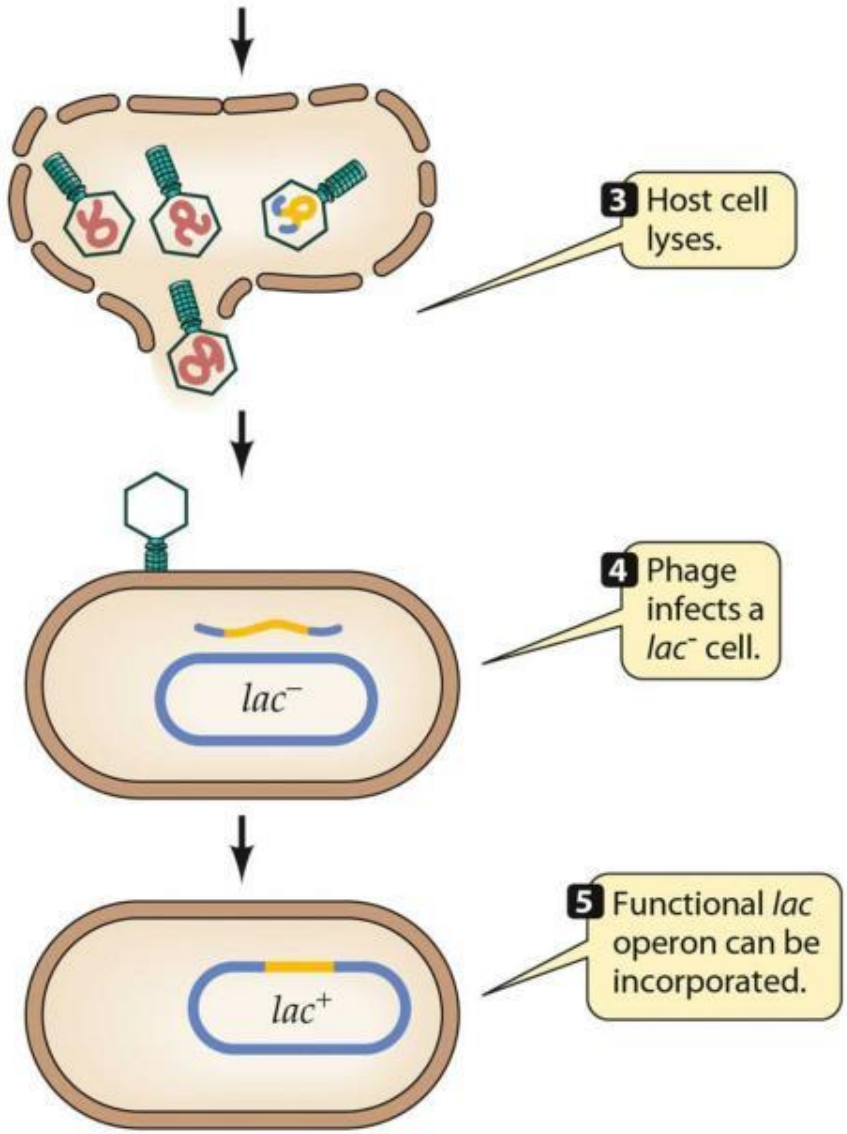
Phages





Generalized transduction: Lytic phage

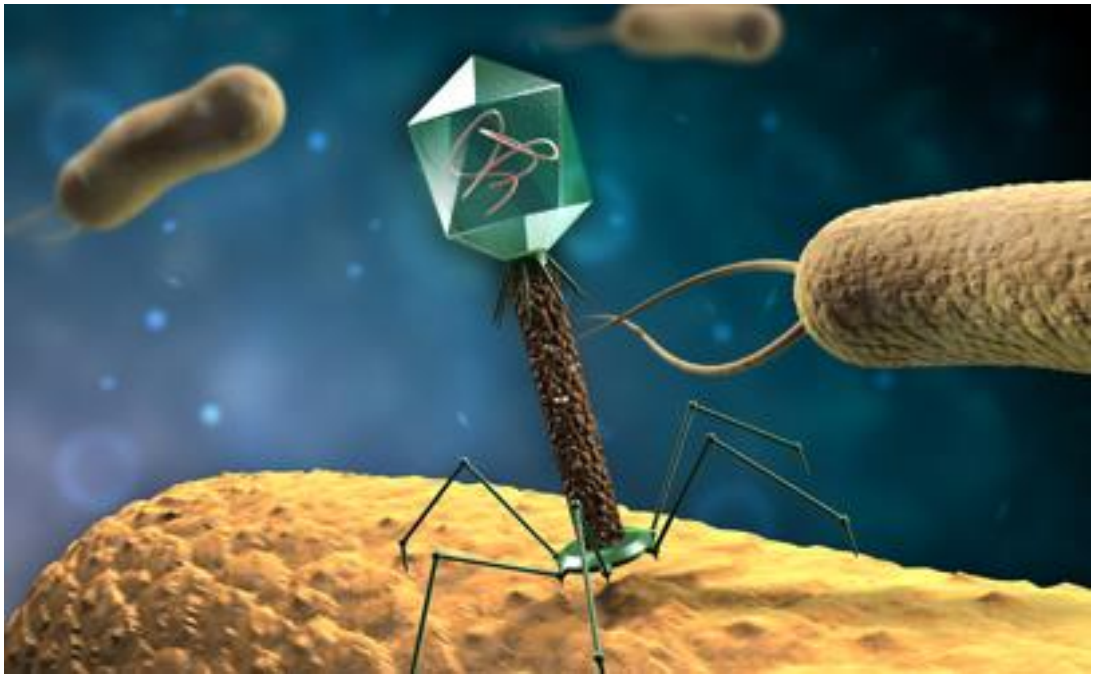




MICROBIAL LIFE, Figure 15.14 (Part 1) © 2002 Sinauer Associates, Inc.

MICROBIAL LIFE, Figure 15.14 (Part 2) © 2002 Sinauer Associates, Inc.

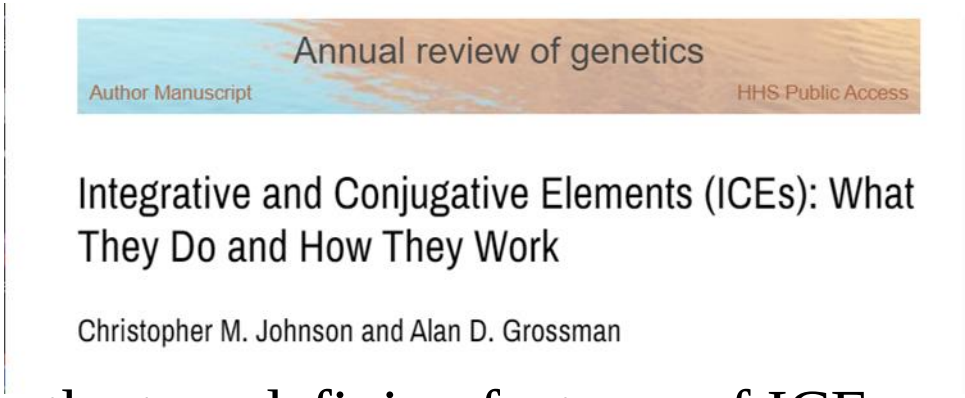
Phage therapy



Danis-Wlodarczyk K, Dąbrowska K, Abedon ST. Phage Therapy: The Pharmacology of Antibacterial Viruses. Curr Issues Mol Biol. 2021;40:81-164. doi: 10.21775/cimb.040.081. Epub 2020 Jun 6. PMID: 32503951.

Causative agent	Model	Condition	Oral	Result summary[1]
Shigella dysenteriae	Human	Dysentery	Oral	All four treated individuals recovered after 24 h
Vibrio cholerae	Human	Cholera	Oral	68 of 73 survived in treatment group and only 44 of 118 in control group
Pseudomonas aeruginosa	Murine	Sepsis	Oral	66.7% reduced mortality
Clostridium difficile	Hamster	Ileocecitis	Oral	Co-administration with *C. difficile* prevented infection
	Hamster	Ileocecitis	Oral	92% reduced mortality
Vancomycin-resistant Enterococcus faecium	Murine	Bacteremia	i.p.	100% reduced mortality
β-*lactamase producing Escherichia coli*	Murine	Bacteremia	i.p.	100% reduced mortality
Imipenem- resistant P. aeruginosa	Murine	Bacteremia	i.p.	100% reduced mortality
Acinetobacter baumannii, P. aeruginosa and *Staphylococcus aureus*	Murine	Sepsis	i.p.	Animals protected against fatal dose of *A. baumannii* and *P. aeruginosa* but not *S. aureus*
Escherichia coli	Murine	Meningitis and Sepsis	i.p.	100% and 50% reduced mortality for meningitis and sepsis, respectively
MDR Vibrio parahaemolyticus	Murine	Sepsis	i.p.	92% and 84% reduced mortality for *i.p*. and oral routes, respectively
S. aureus	Rabbit	Wound infection	s.c.	Co-administration with *S. aureus* prevented infection
MDR S. aureus	Human	Diabetic foot ulcer	Topical	All 6 treated patients recovered
Unclassified bacterial dysentery	Human	Dysentery	Oral	Phage cocktail improved symptoms of 74% of 219 patients
Salmonella typhi	Human	Typhoid	Oral	In cohort of 18577 children, phage treatment associated with 5-fold decrease in typhoid incidence compared to placebo
Antibiotic-resistant *P. aeruginosa*	Human	Chronic Otitis	Oral	Phage treatment safe and symptoms improved in double-blind, placebo-controlled Phase I/II trial

Integrative and Conjugative Elements (ICEs)



the two defining features of ICEs are that they integrate into the host genome and that they encode a functional conjugation system that mediates their intercellular transfer.

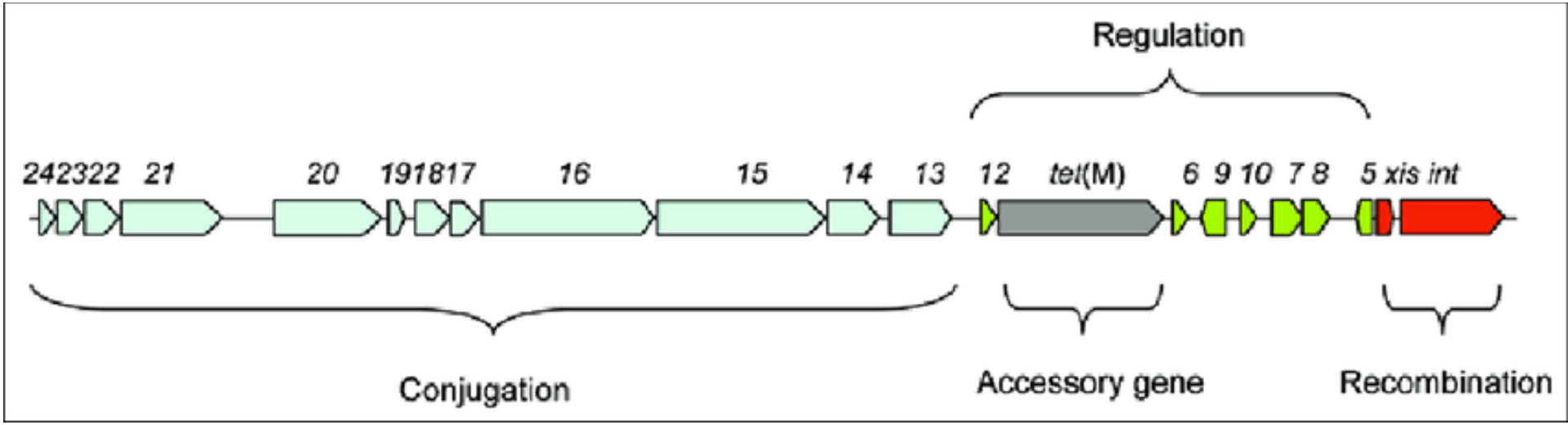
Induction of ICE gene expression leads to excision, production of the conserved conjugation machinery (a type IV secretion system), and the potential to transfer DNA to appropriate recipients.

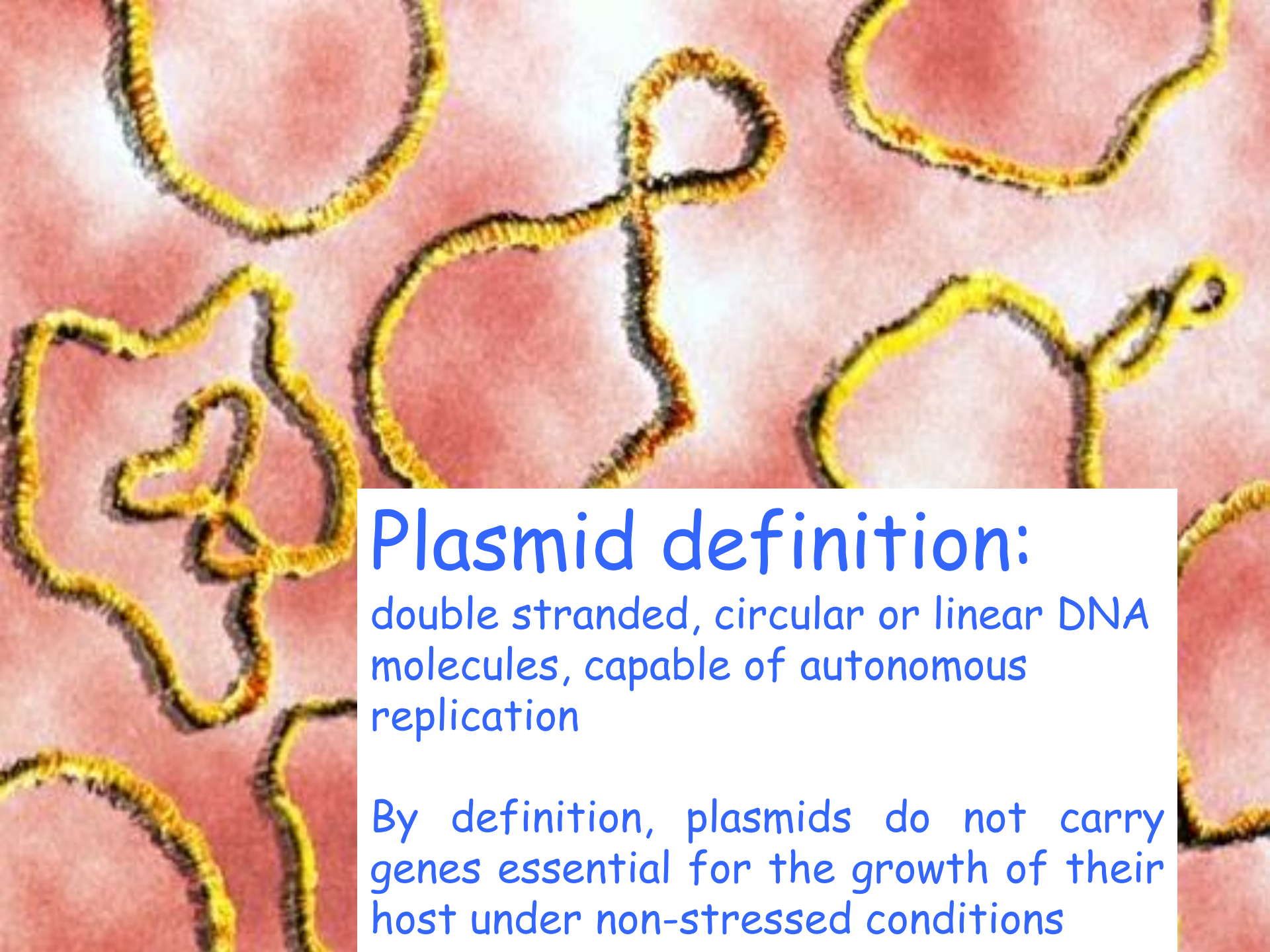
ICEs typically contain cargo genes that are not usually related to the ICE life cycle and that confer phenotypes to host cells.

DNA damaging agents cause induction of the recA dependent SOS response in host cells and also induce several ICEs. During the SOS response, DNA damage generates ssDNA. This is bound by and activates RecA, which causes auto-cleavage of repressors.

Size range: approximately 18 kb (Tn*916*) to more than 500 kb (ICE*Ml*Sym^{R7A}).
Some phenotypes conferred by ICEs: antibiotic resistance(s)

Tn*916*



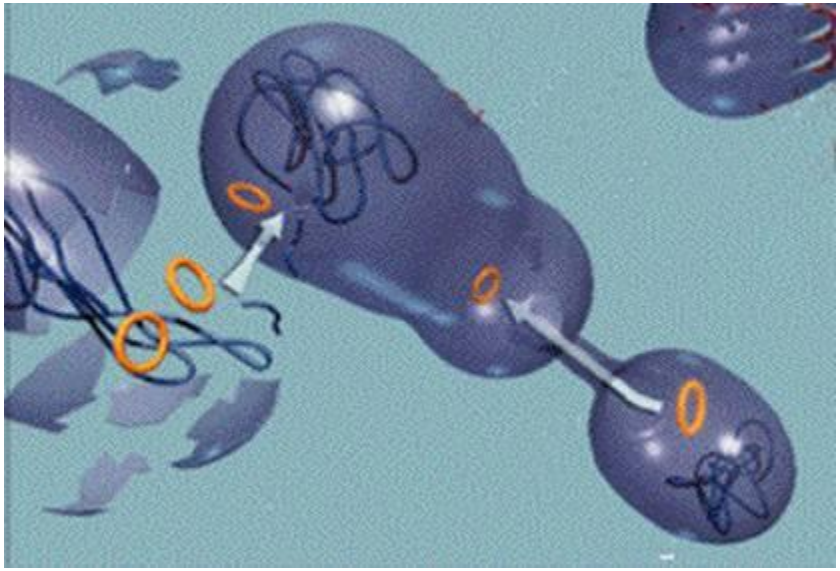


Plasmid definition:

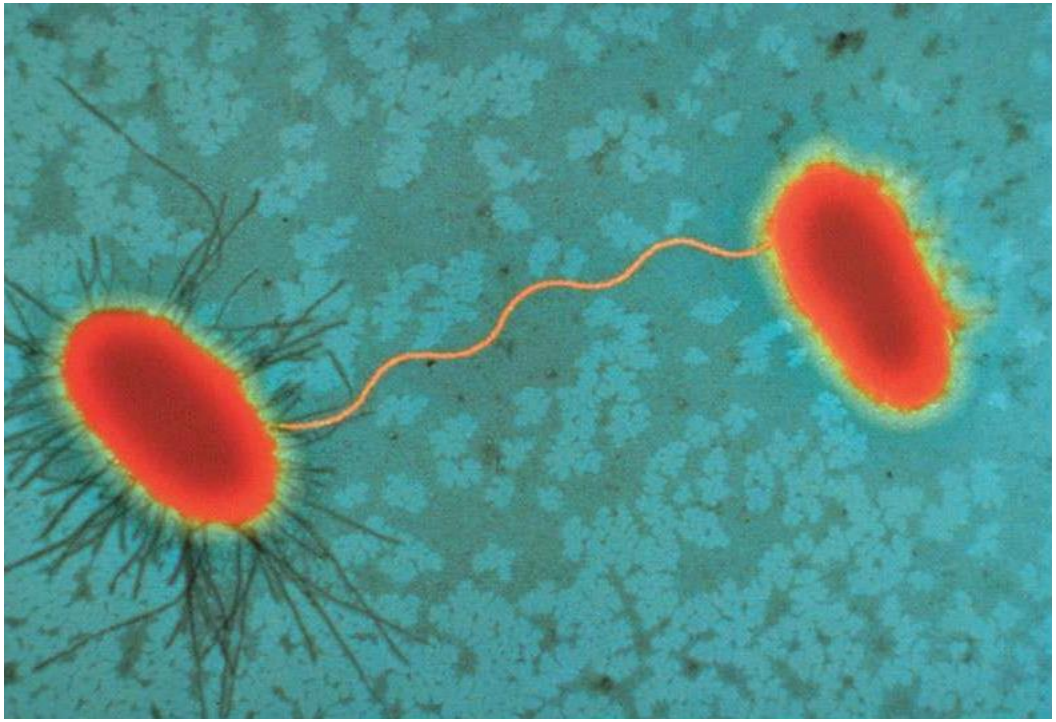
double stranded, circular or linear DNA molecules, capable of autonomous replication

By definition, plasmids do not carry genes essential for the growth of their host under non-stressed conditions

Plasmids promote their diffusion

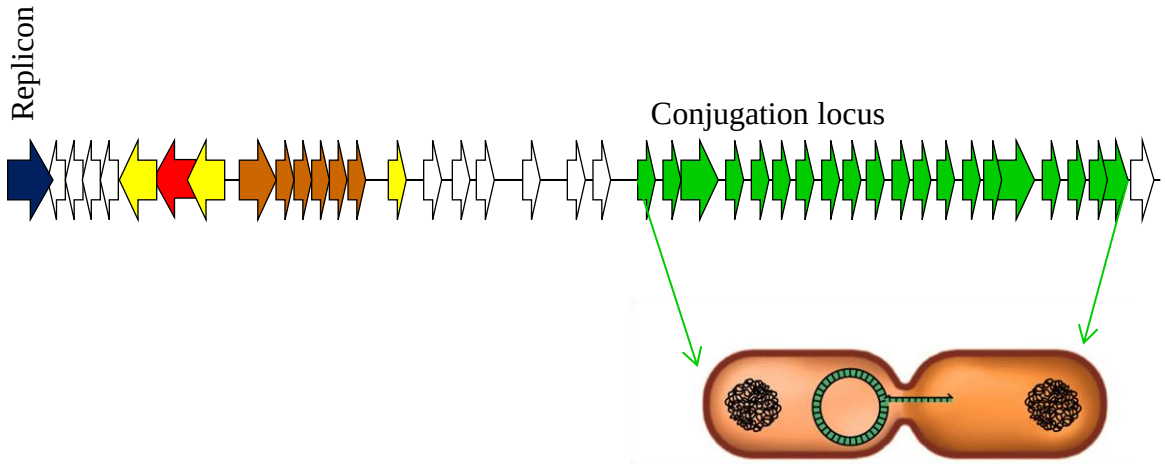


Coniugation



Sexual pili: present in numbers of 1-10 per cell, they are 9-10 nm thick

Plasmids



replication stability conjugation resistance Mobile elements other

Review Article | Published: 09 October 2023

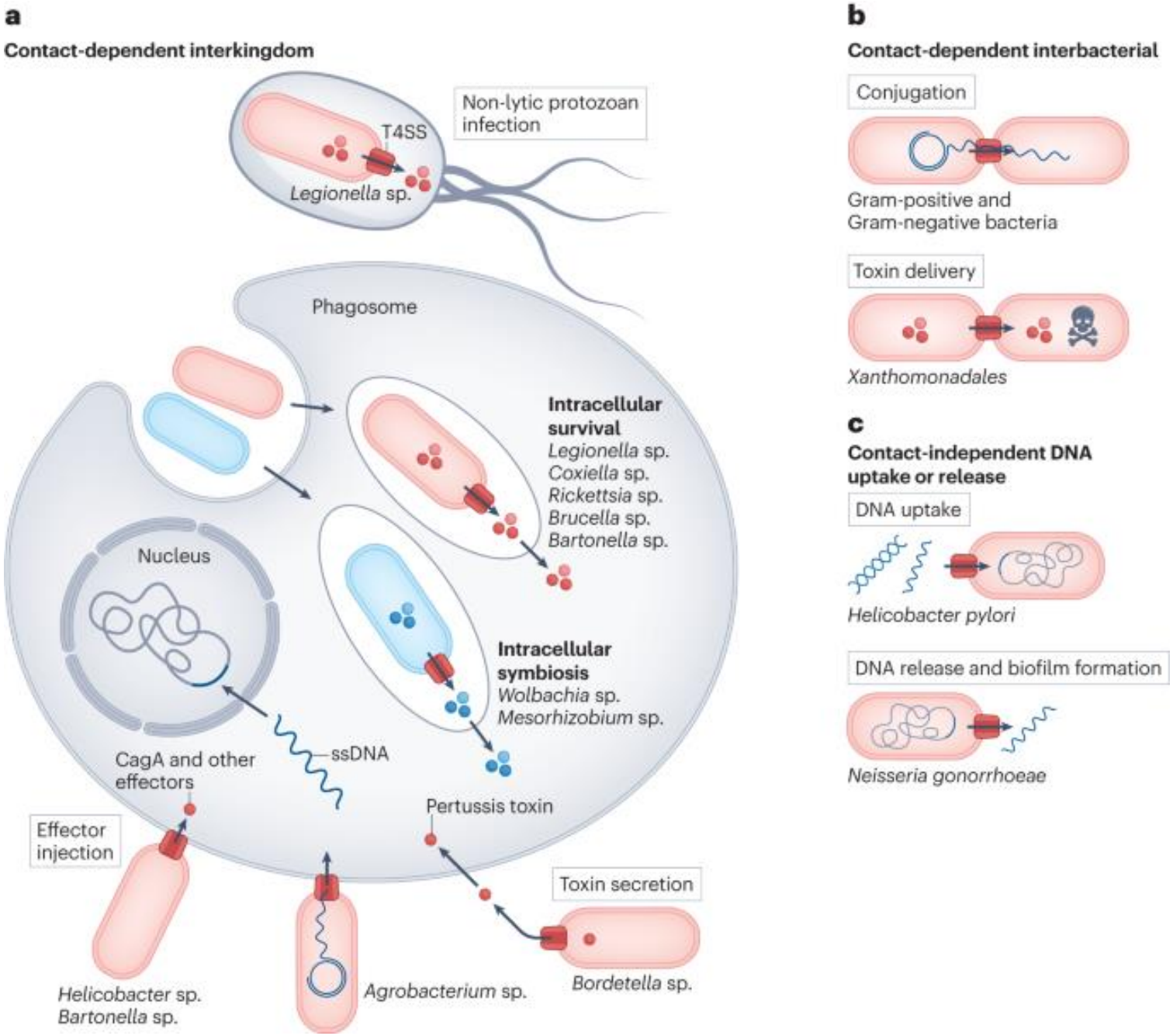
Structural and functional diversity of type IV secretion systems

Tiago R. D. Costa, Jonasz B. Patkowski, Kévin Macé, Peter J. Christie & Gabriel Waksman

Nature Reviews Microbiology **22**, 170–185 (2024) | Cite this article

Abstract

Considerable progress has been made in recent years in the structural and molecular biology of type IV secretion systems in Gram-negative bacteria. The latest advances have substantially improved our understanding of the mechanisms underlying the recruitment and delivery of DNA and protein substrates to the extracellular environment or target cells. In this Review, we aim to summarize these exciting structural and molecular biology findings and to discuss their functional implications for substrate recognition, recruitment and translocation, as well as the


a
Contact-dependent interkingdom
Non-lytic protozoan infection
T4SS
Legionella sp.
Phagosome
Intracellular survival
Legionella sp.
Coxiella sp.
Rickettsia sp.
Brucella sp.
Bartonella sp.
Nucleus
Intracellular symbiosis
Wolbachia sp.
Mesorhizobium sp.
ssDNA
CagA and other effectors
Pertussis toxin
Effector injection
Toxin secretion
Helicobacter sp.
Bartonella sp.
Agrobacterium sp.
Bordetella sp.
b
Contact-dependent interbacterial
Conjugation
Gram-positive and Gram-negative bacteria
Toxin delivery
Xanthomonadales
c
Contact-independent DNA uptake or release
DNA uptake
Helicobacter pylori
DNA release and biofilm formation
Neisseria gonorrhoeae

Various pathogenic bacteria and symbionts deploy **type IV secretion systems (T4SSs)** to deliver effector proteins, DNA–protein complexes or other macromolecules into eukaryotic or protozoan host cells.

a, The T4SS establishes contact-dependent interkingdom interactions by injecting effectors directly into eukaryotic cells to promote bacterial intracellular survival and symbiosis.

b, Many bacterial species and a few Archaea deploy a contact-dependent T4SS for the delivery of DNA and toxins to other bacteria or Archaea. Various species in the *Xanthomonadales* instead deploy T4SSs for the contact-dependent delivery of protein toxins to kill other bacteria for niche establishment.

c, Some bacteria can deploy T4SSs for the contact-independent uptake or release of DNA. ssDNA, single-stranded DNA.

Type IV Secretion System: DNA secretion and HGT

- Plasmids
- Integrative Conjugative Elements ICE
- Conjugative-Transposons

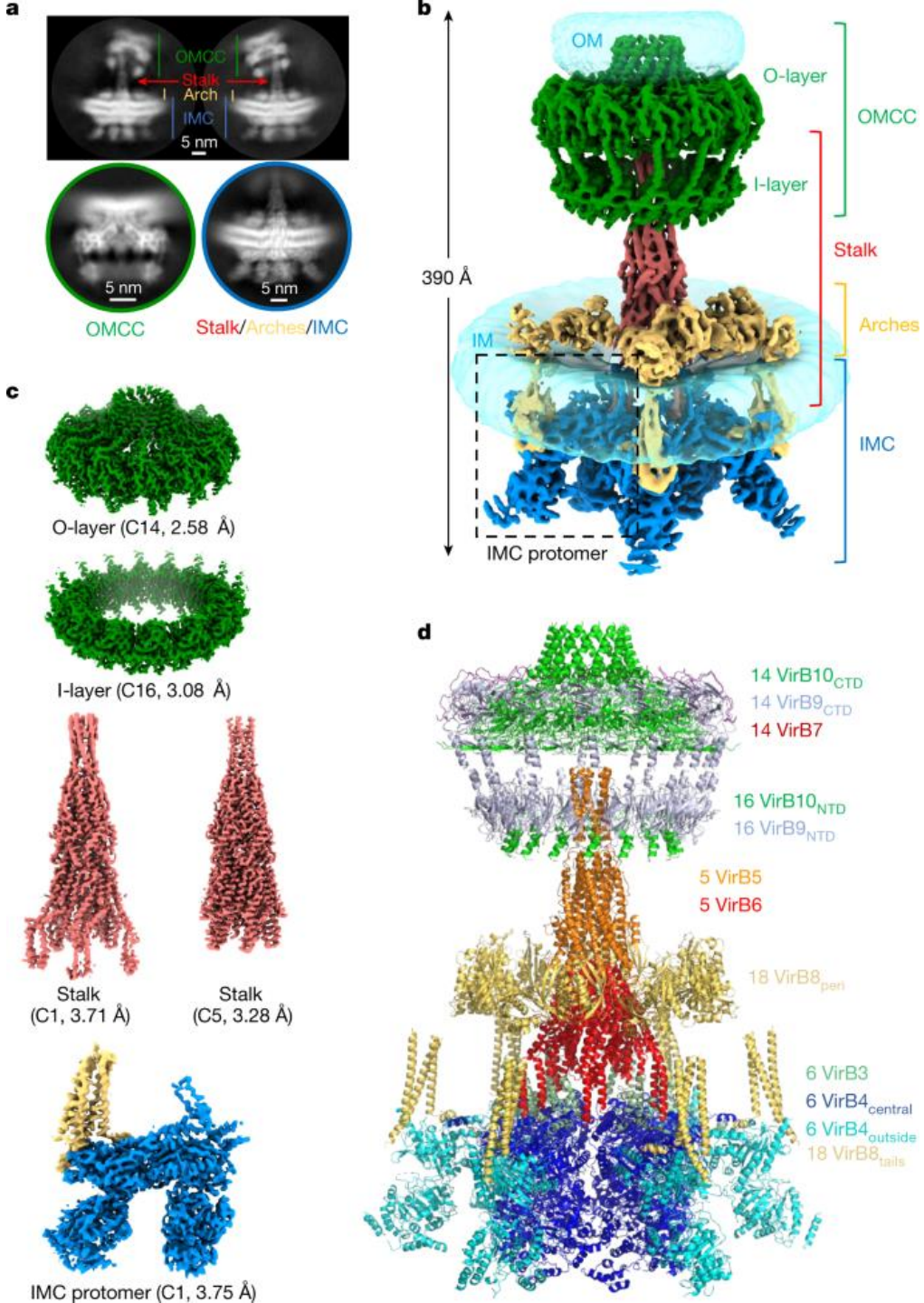
Table 1. PubMed Search Performed on the 15 April 2020, Including 'Plasmid', 'Integrative Conjugative Element', or the Former Designation 'Conjugative Transposon' and a Combination of Relevant Keywords

	Evolution	Antibiotic resistance	Fitness	Compensatory
Plasmid	7550	18 720	738	220
Integrative conjugative element	278	403	36	3
Conjugative transposon	441	1343	54	4

T4SSs in Gram-negative species are composed minimally of 12 core subunits that are generically termed VirB1–VirB11 and VirD4 (ref. [8]). Systems assembled only with the core VirB–VirD4 components are considered ‘minimized’, and many of these systems function as conjugation machines by delivering DNA substrates to target bacteria[9,10]. Over the course of evolution, T4SSs have acquired several additional protein components that are integrated into the core structure composed of VirB and VirD4 proteins. As a result, assembly of an expanded T4SS may require up to 25 different proteins[10,11]. Some of these expanded systems can mediate conjugative DNA transfer, but many have acquired new functionalities relating to translocation of effector proteins or toxins, with or without retention of the ancestral DNA transfer function[12,13].

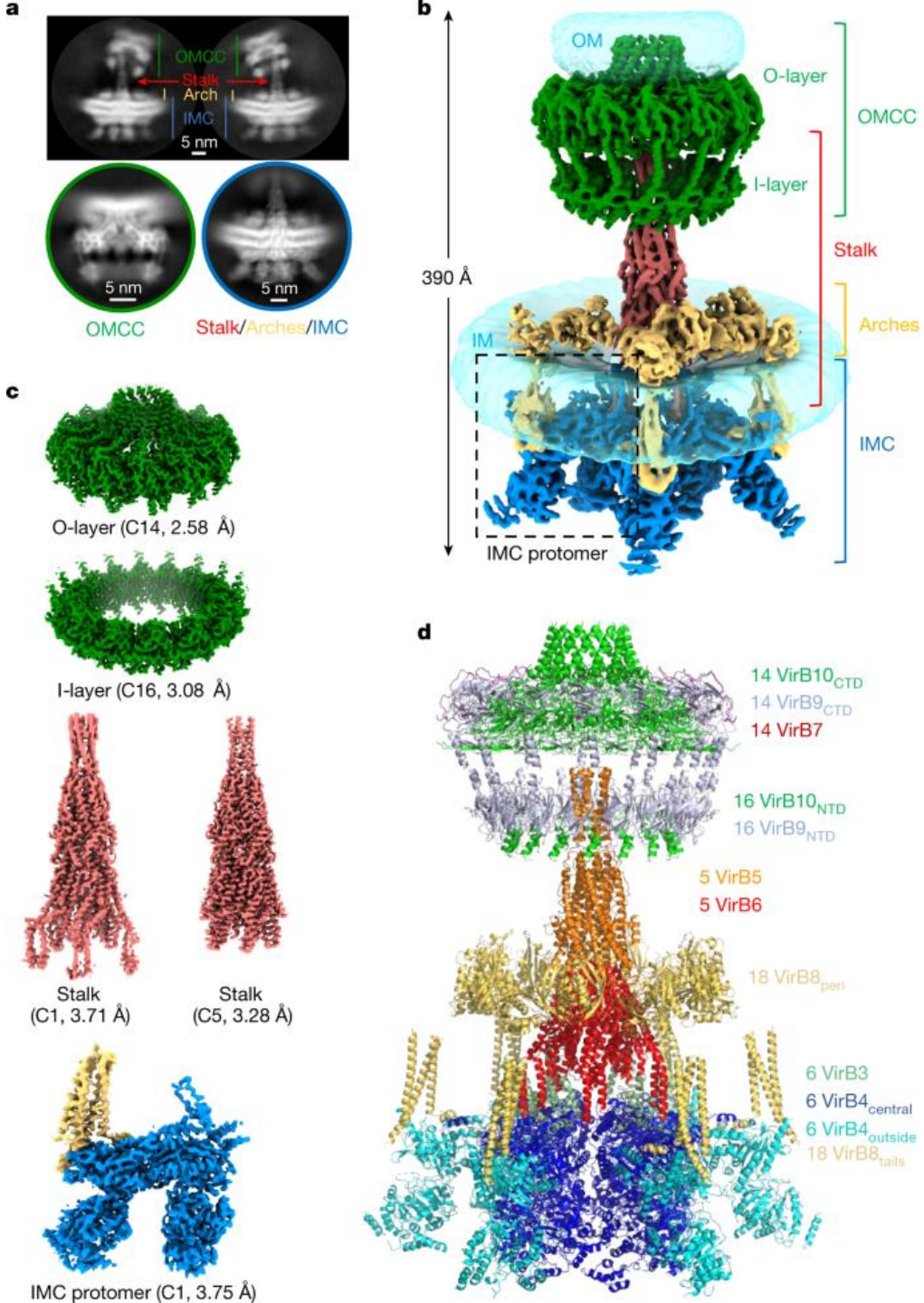
Early biochemical studies supplied evidence that the VirB subunits VirB7, VirB9 and VirB10 assemble as a stabilizing structural scaffold for the T4SS; this scaffold ultimately was designated as the **outer membrane core complex (OMCC)**[8,15].

The most recent structure presented for the nearly intact T4SS encoded by plasmid R388 ($T4SS_{R388}$) now has provided important refinements of these earlier structures

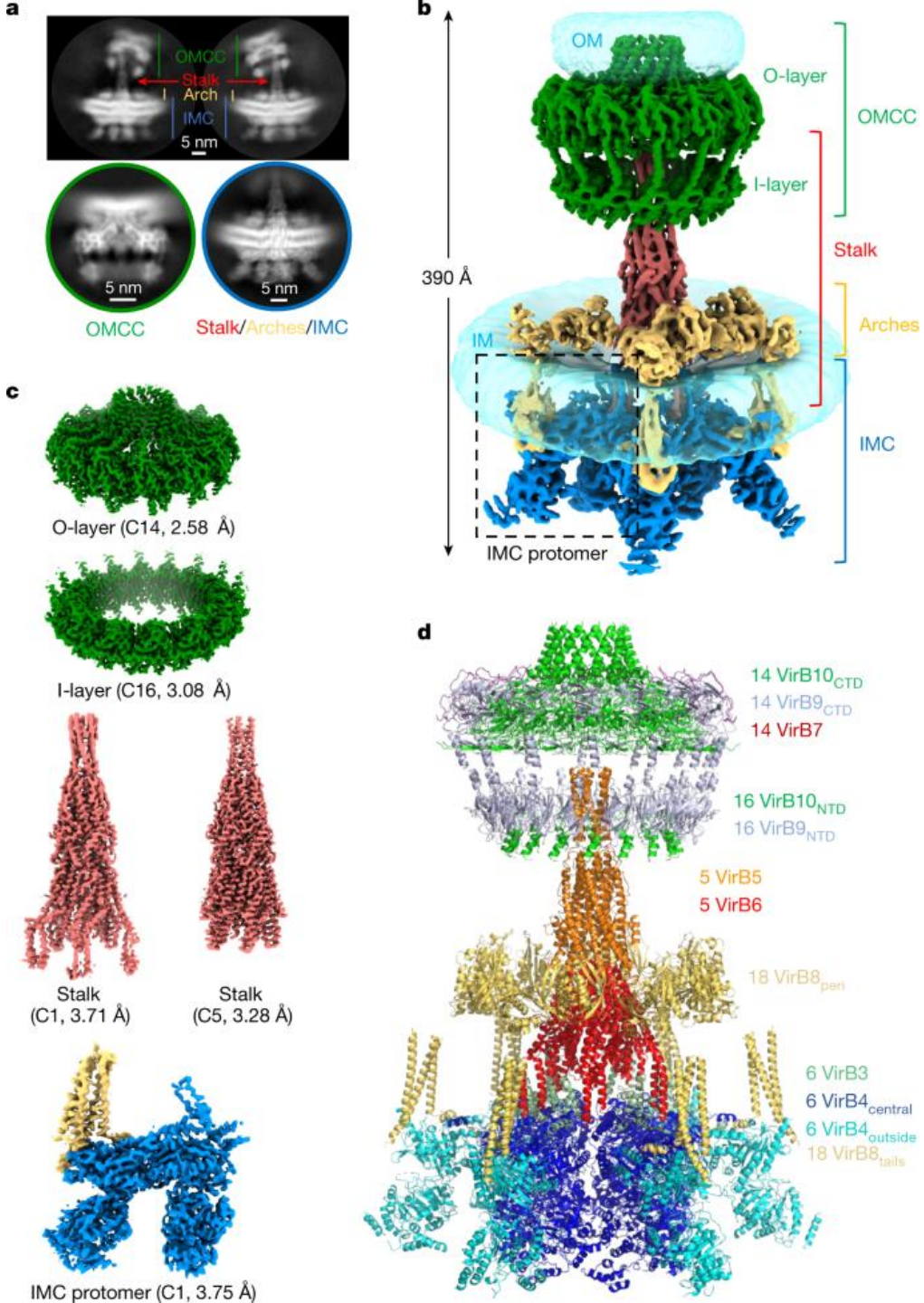


Here we present a single-particle cryo-EM structure of a T4SS complex from the R388 plasmid that comprises all four sub-complexes: OMCC, stalk, arches and IMC

Three proteins, VirB7 (also known as TrwH), VirB9 (also known as TrwF) and VirB10 (also known as TrwE), form the outer membrane core complex (OMCC), which contains an O-layer embedded in the outer membrane and an I-layer underneath[7]

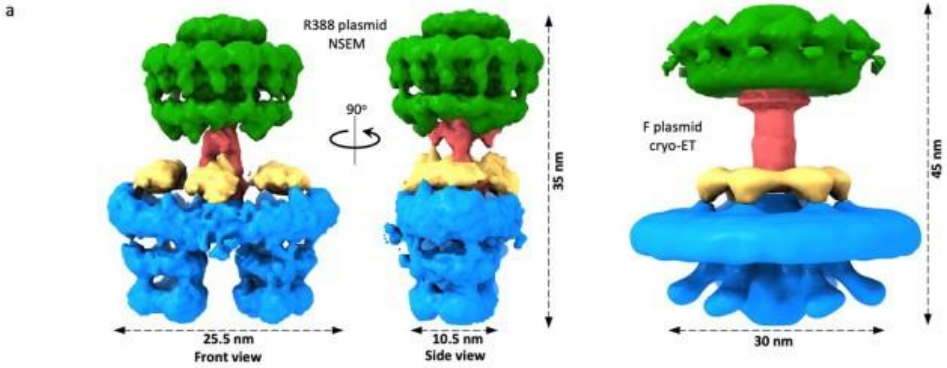


The other proteins (except VirB2 (also known as TrwL), which forms the conjugative pilus and VirB5 (also known as TrwJ), which locates at the tip of the pilus) assemble to form three additional sub-complexes. These sub-complexes consist of an inner membrane complex (IMC) embedded in the inner membrane, a structure bridging the OMCC and the IMC (the stalk (also called the cylinder), and a ring complex surrounding the stalk (the arches)



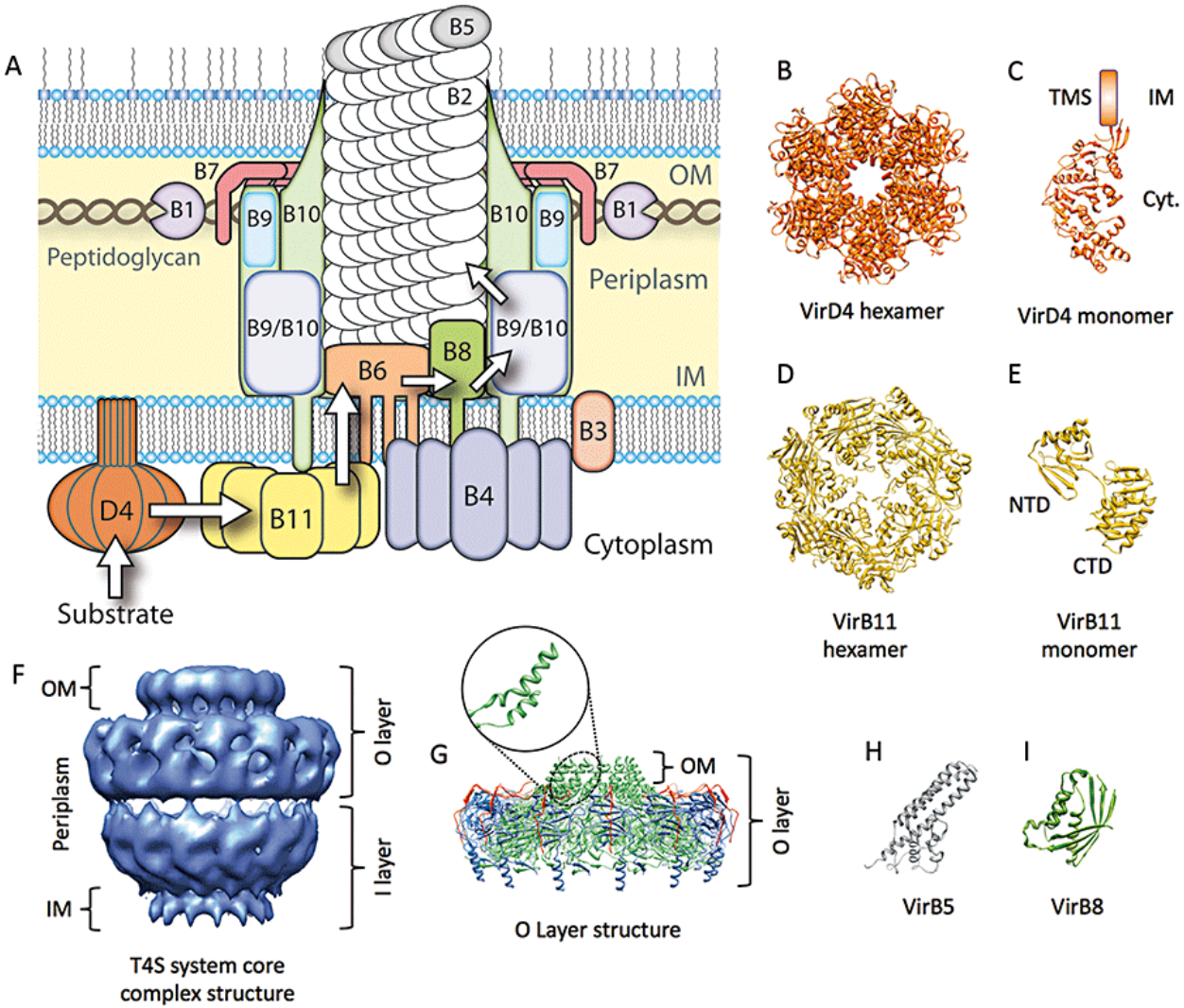
Conjugative T4SSs must first produce a conjugative pilus, which makes contact with a recipient cell[12] and may serve as a conduit for DNA[13]. In this pilus biogenesis mode, only the VirB2–VirB11 proteins are required[14,15].

After contact between cells is made, the T4SS switches to a DNA-transfer mode involving VirB2–VirB11 and VirD4

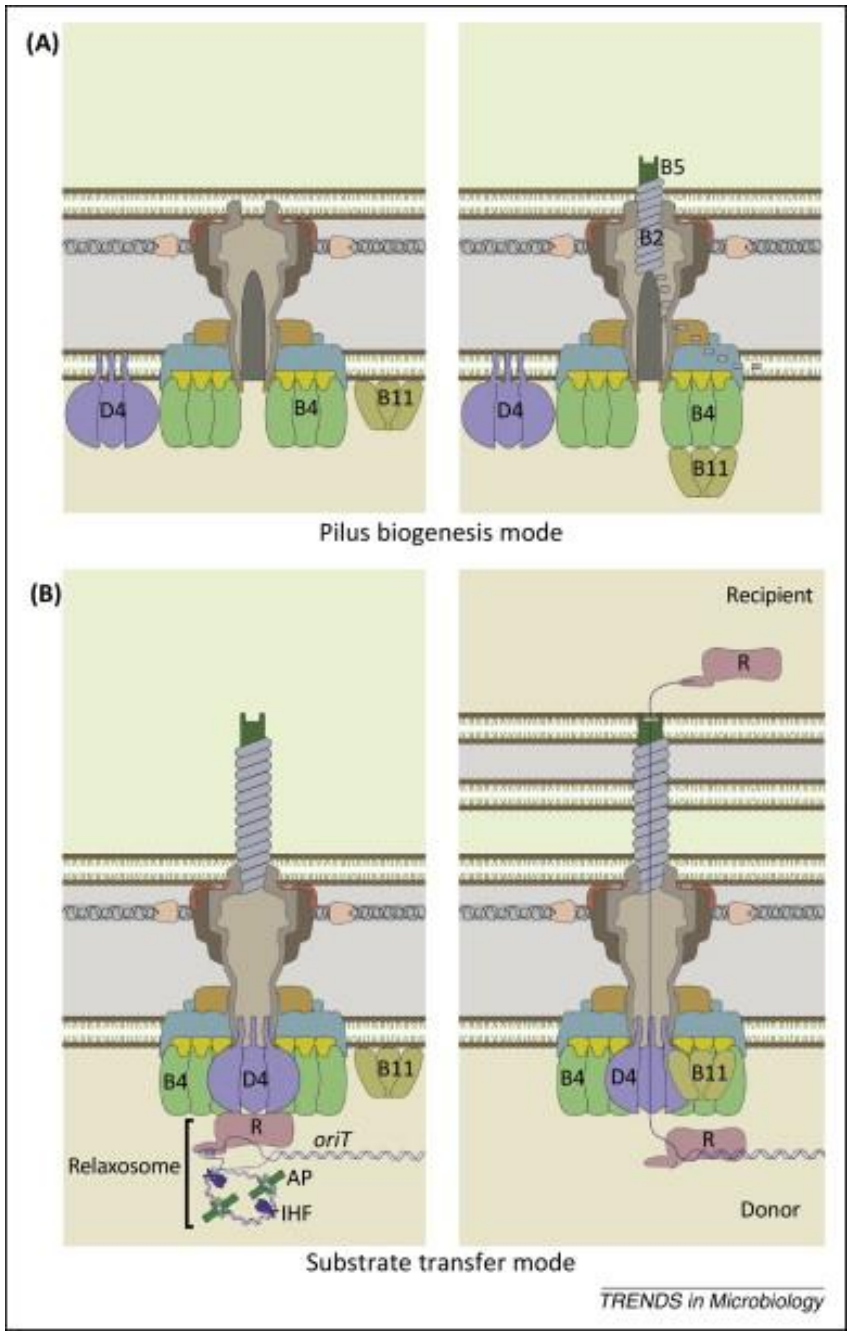


Protein	Built with side chain	Main chain backbone only	Missing
TrwM/VirB3	72% (75)	28 % (29)	-
TrwK/VirB4	84 % (689)	7 % (60)	9 % (74)
TrwJ/VirB5	96 % (197)	-	4 % (9)
TrwI/VirB6	63 % (214)	14 % (47)	23 % (81)
TrwH/VirB7	100 % (27)	-	-
TrwG/VirB8	22% (50)	59% (136)	19% (45)
TrwF/VirB9	100 % (245)	-	-
TrwE/VirB10	52 % (207)	-	48 % (188)

Architecture of Type IV secretion system (T4SS)

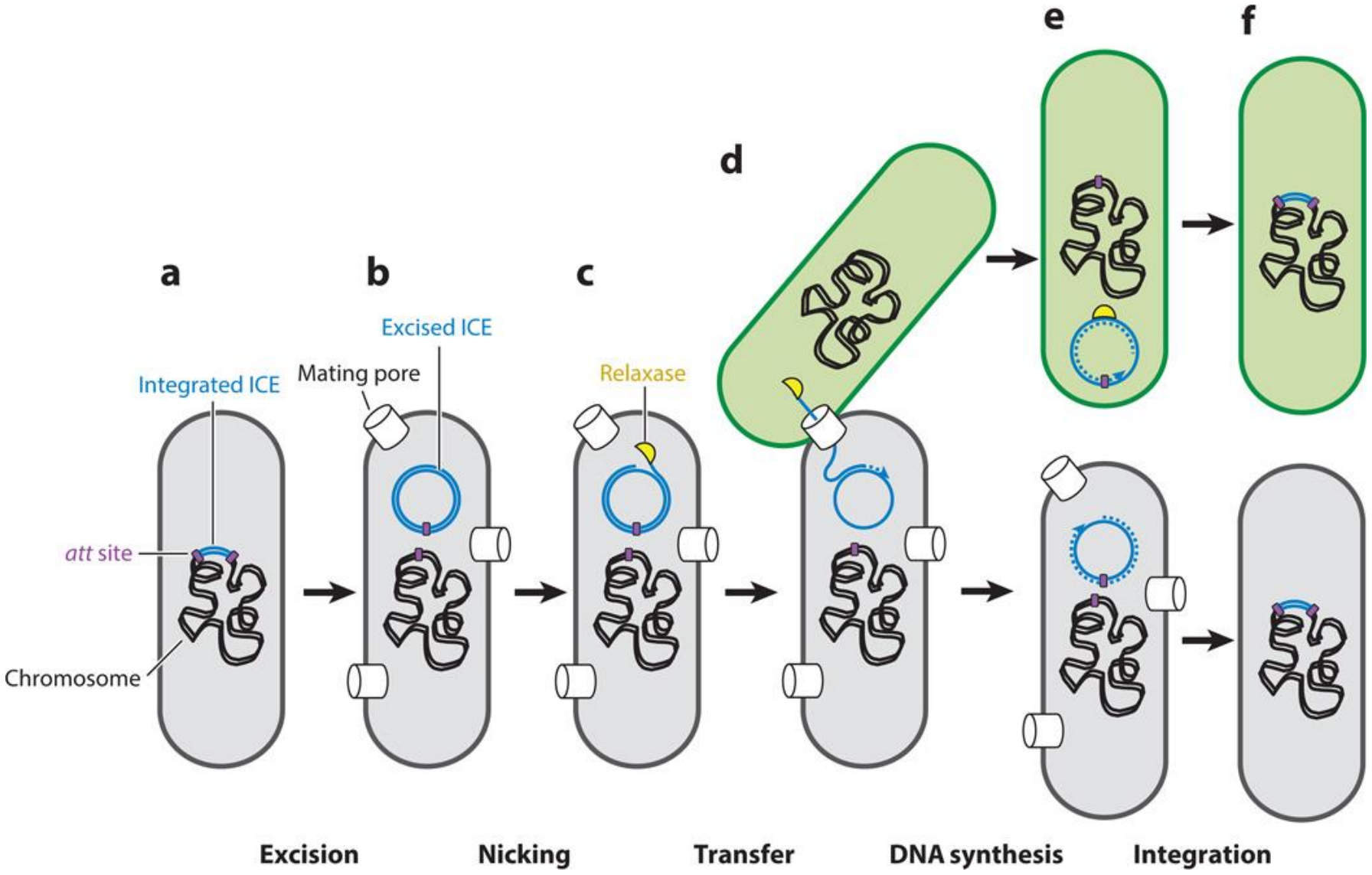


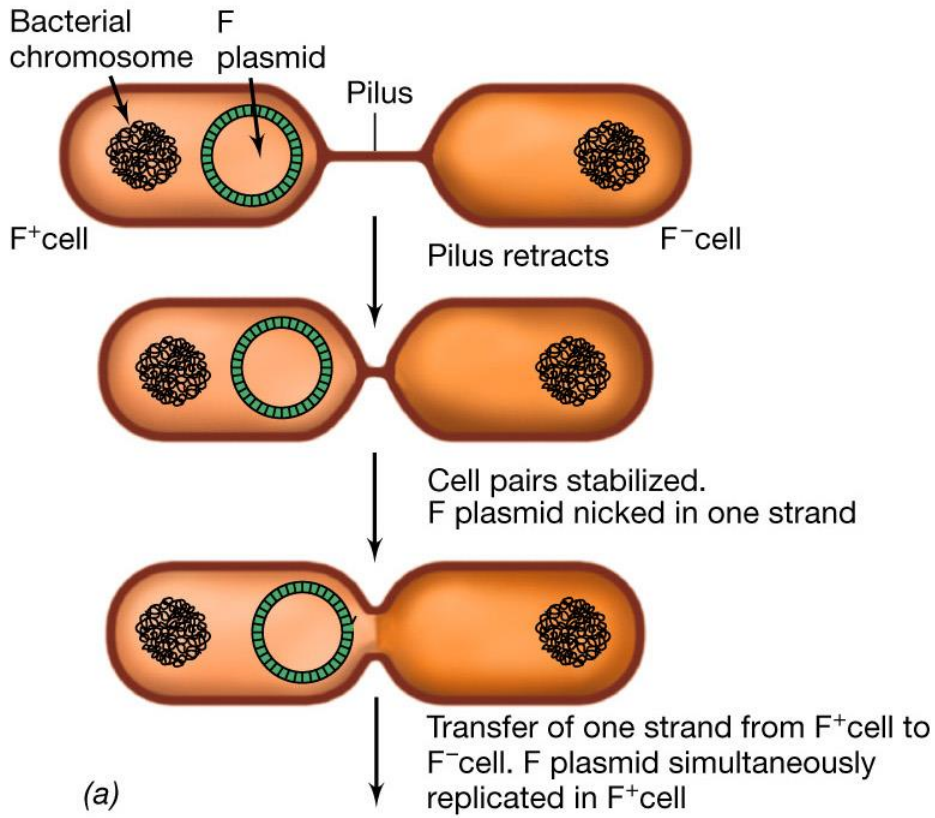
Wallden et al., 2010 Microreview: Type IV secretion systems: versatility and diversity in function. Cellular Microbiology 12: 1203-1212



(A) The pilus biogenesis mode showing the pilus growing from the stalk structure within the T4SS. At this stage VirB11 (light brown) interacts with VirB4 (green) to activate this mode.
(B) A substrate translocation mode where VirB11 (light brown) interacts with VirD4 (purple) facilitating substrate transfer.
The relaxosome [relaxase (R); accessory protein (AP); origin of transfer (*oriT*) DNA; and integration host factor (IHF)] processes the DNA and is recruited to the T4SS through interactions with the VirD4 coupling protein (left panel). This is followed by the transfer of both the DNA and the relaxase to the recipient cell (right panel).

Ilangovan et al., 2015 Structural biology of the Gram-negative bacterial conjugation systems, Trends in Microbiology, 23:301–310


a
b
c
d
e
f
Integrated ICE
Excised ICE
Mating pore
Relaxase
att site
Chromosome
Excision
Nicking
Transfer
DNA synthesis
Integration


Bacterial chromosome
F plasmid
Pilus
F+cell
F−cell
Pilus retracts
Cell pairs stabilized.
F plasmid nicked in one strand
Transfer of one strand from F+cell to F−cell. F plasmid simultaneously replicated in F+cell
(a)

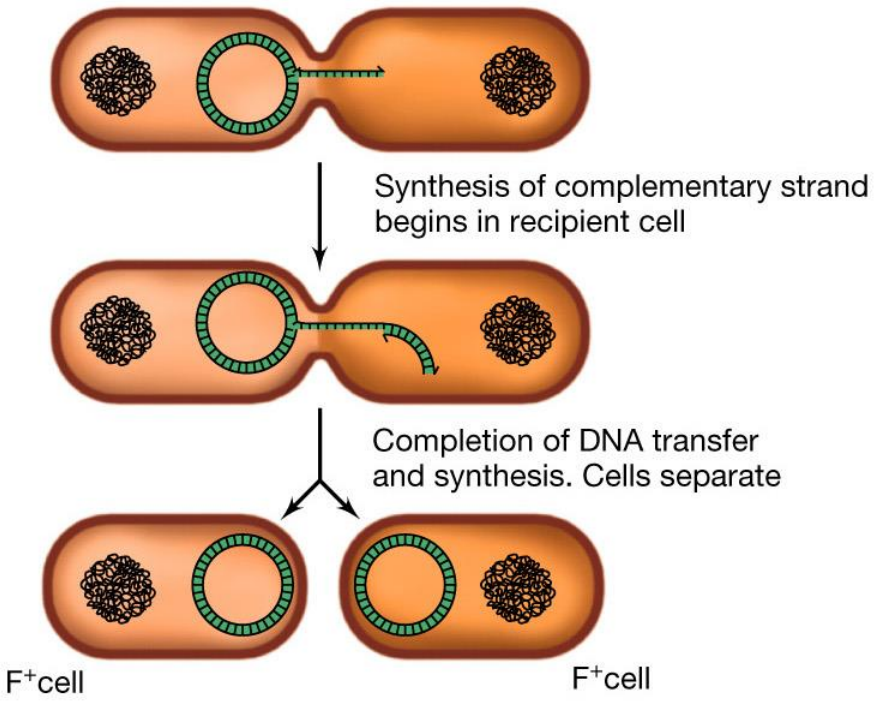
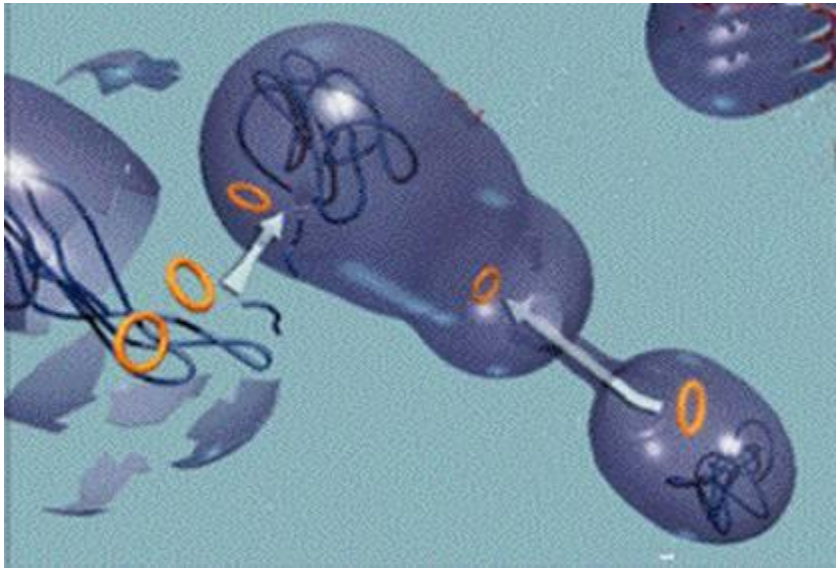

Synthesis of complementary strand begins in recipient cell
Completion of DNA transfer and synthesis. Cells separate
F+cell
F+cell

Table 2. Specific Features Typically Associated with ICEs and Conjugative Plasmids

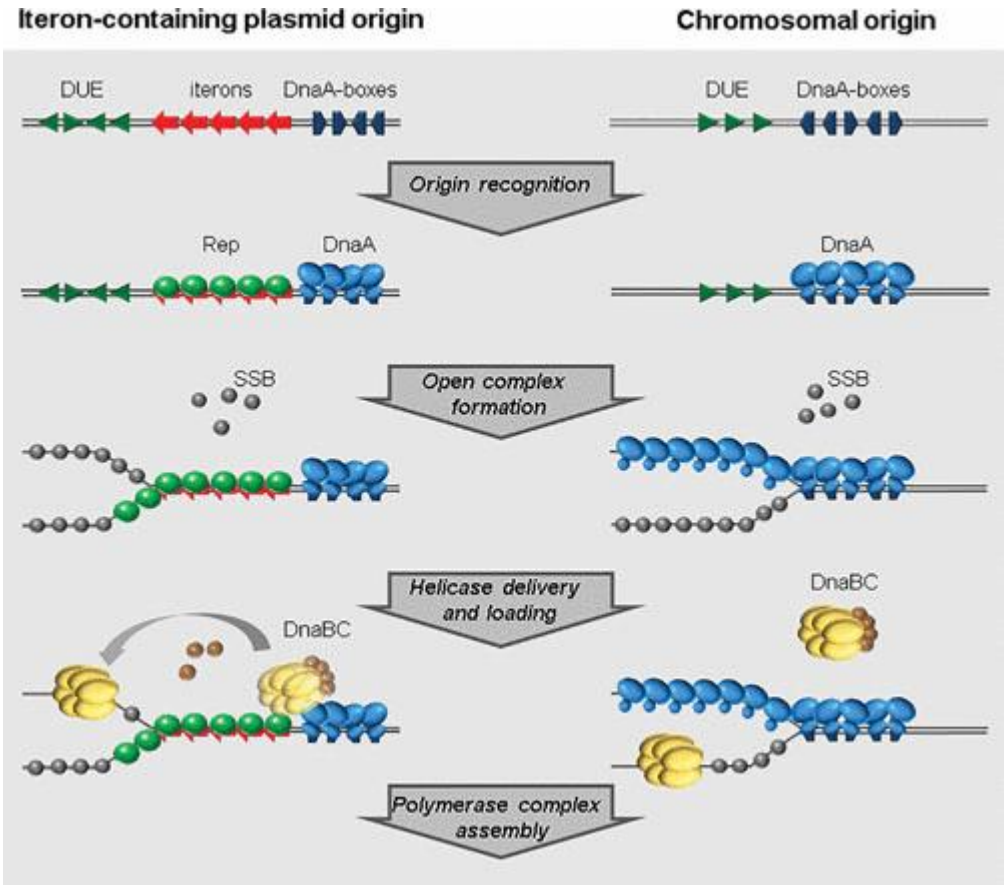
	ICEs	Conjugative plasmids
What separates them		
Location	Integrated in the chromosome[a]	Extrachromosomal
Signature modules[b]	Integration/excision	Replication
GC content (by comparison with that of the host genome)[c]	Closer	More distinct
Size[c]	Less variable	More variable
Density of DNA repeats[c]	Lower	Higher
What brings them together		
Type of mobility	Intercellular	
Mobility mechanism	Conjugation	
Shared modules	Maintenance, conjugative transfer	

[a]ICEs can also exist as circular extrachromosomal elements, formed upon excision and transfer to a new host.
[b]Even though the integration/excision module is classically associated with ICEs and the replication module with plasmids, ICEs may carry genes coding for replicases, while some plasmids may also carry genes encoding integrases [27].
[c]Data retrieved from the comparison between conjugative plasmids and ICEs belonging to a specific mating-pair formation class, the MPF_T [27].

Plasmids control the initiation of replication independently by the replication of the bacterial chromosome

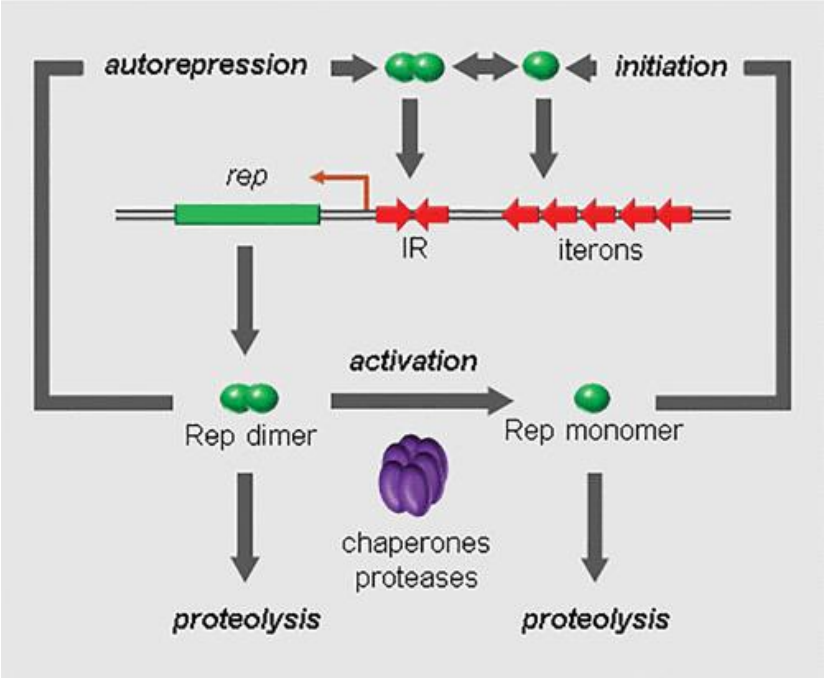


IN CIS ELEMENTS: ITERONS



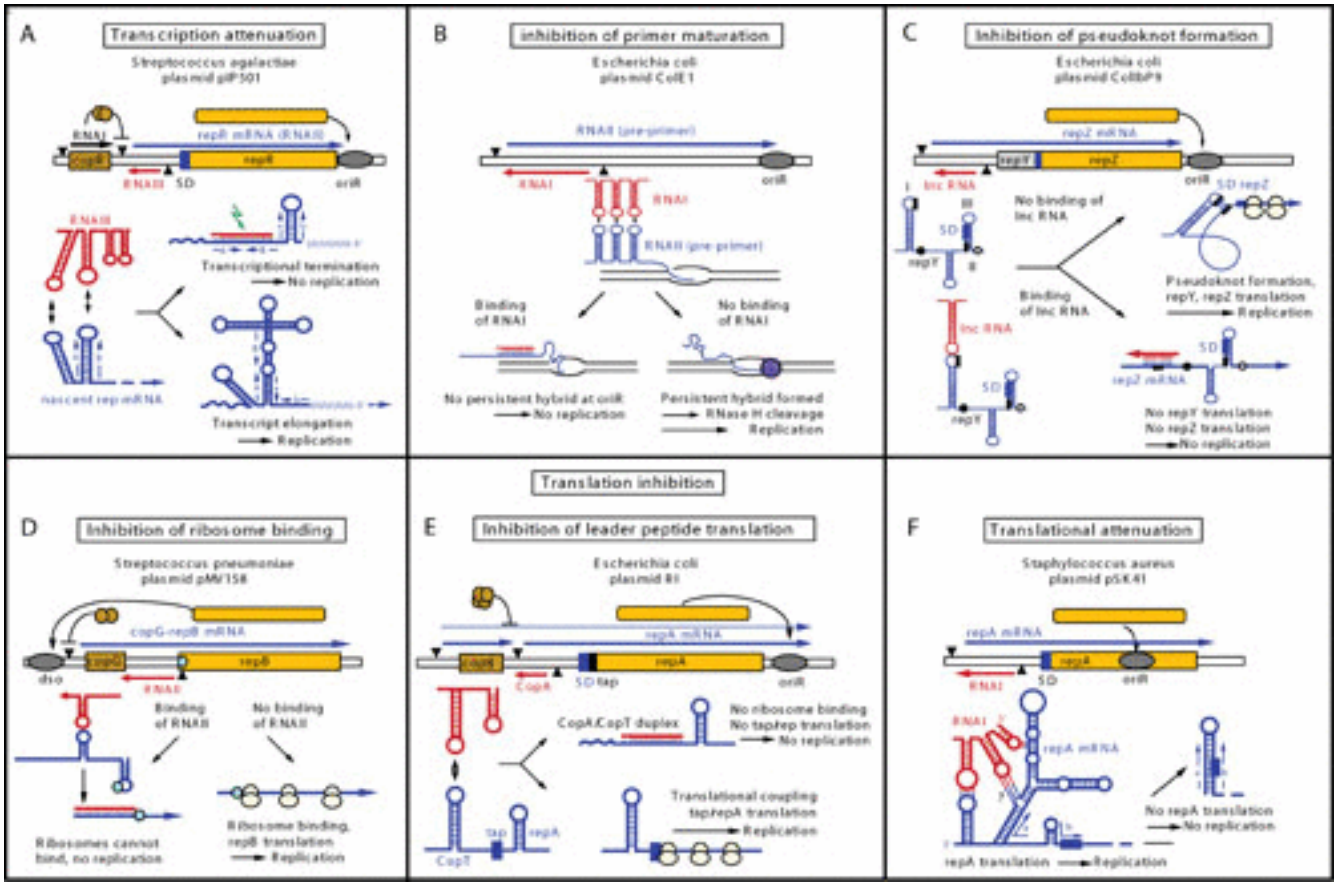
The iteron-containing plasmid origin is recognized by the plasmid-encoded initiator (Rep), which binds cooperatively to the iterons.
plasmid Rep +host DnaA proteins, while at the chromosomal origin the DnaA protein is sufficient for this process.
Rep translocates the DnaBC helicase to the opened plasmid origin.

Konieczny et al., S., Microbiol Spectr. 2014 ;2(6)



Regulation of iteron-containing plasmid replication initiation by the auto-repression mechanism. Binding of Rep dimers to inverted repeats inhibits the initiation of transcription starting from the *rep* gene promoter. Proteases limit the amount of both dimer and monomer forms of the Rep protein.

ANTISENSE RNAS



Brantl S., Microbiol Spectr. 2014 ;2(4)

- CopR represses transcription from the *repR* promoter
- Binding of Inc RNA to the *repZ* RNA inhibits formation of the pseudoknot and inhibits *repY* translation
- Translation inhibition by inhibition of ribosome binding.
- The CopB protein represses transcription from the *repA* promoter
- The antisense RNA interacts via three loops with the nascent *repA* mRNA resulting in a stem-loop structure that sequesters the ribosome binding site

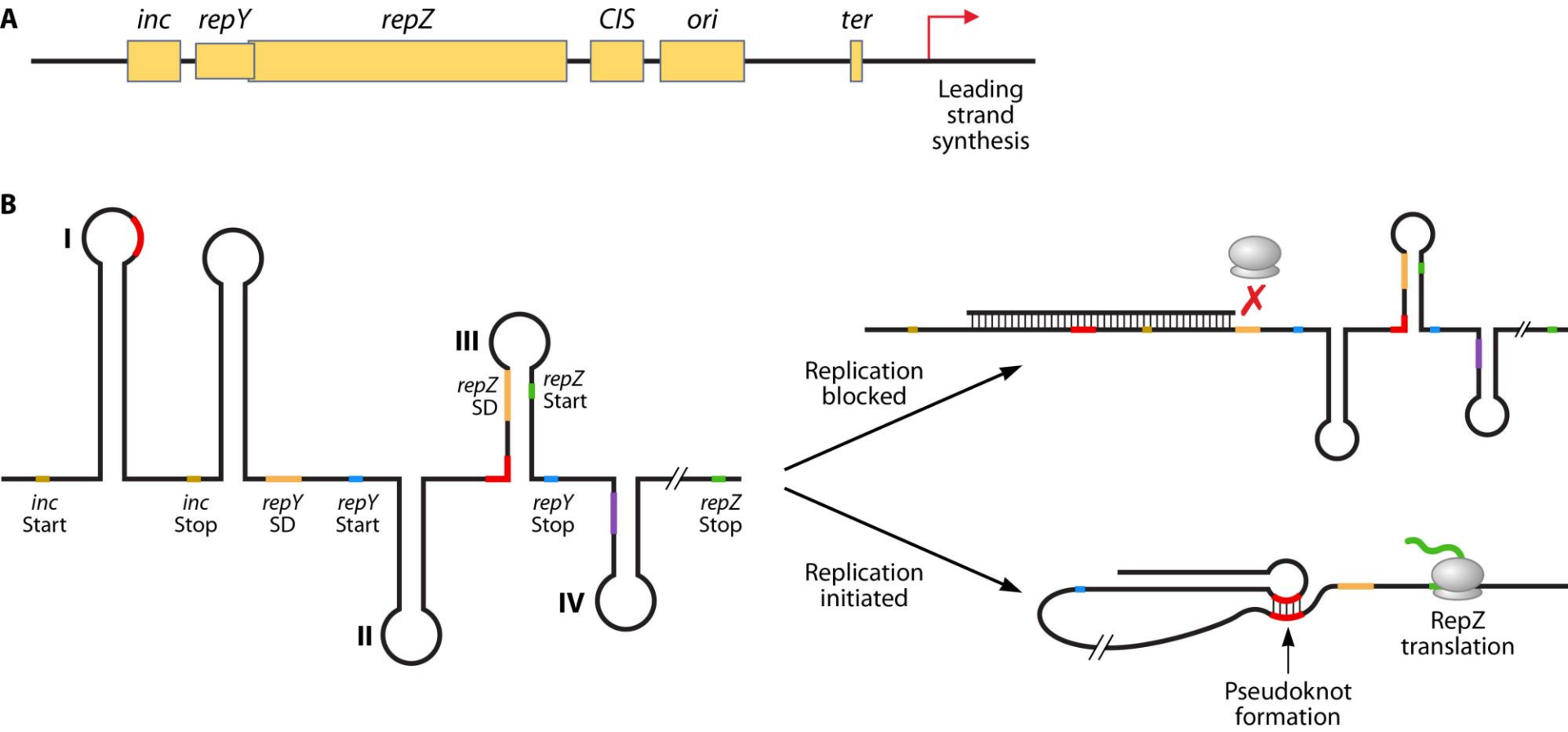
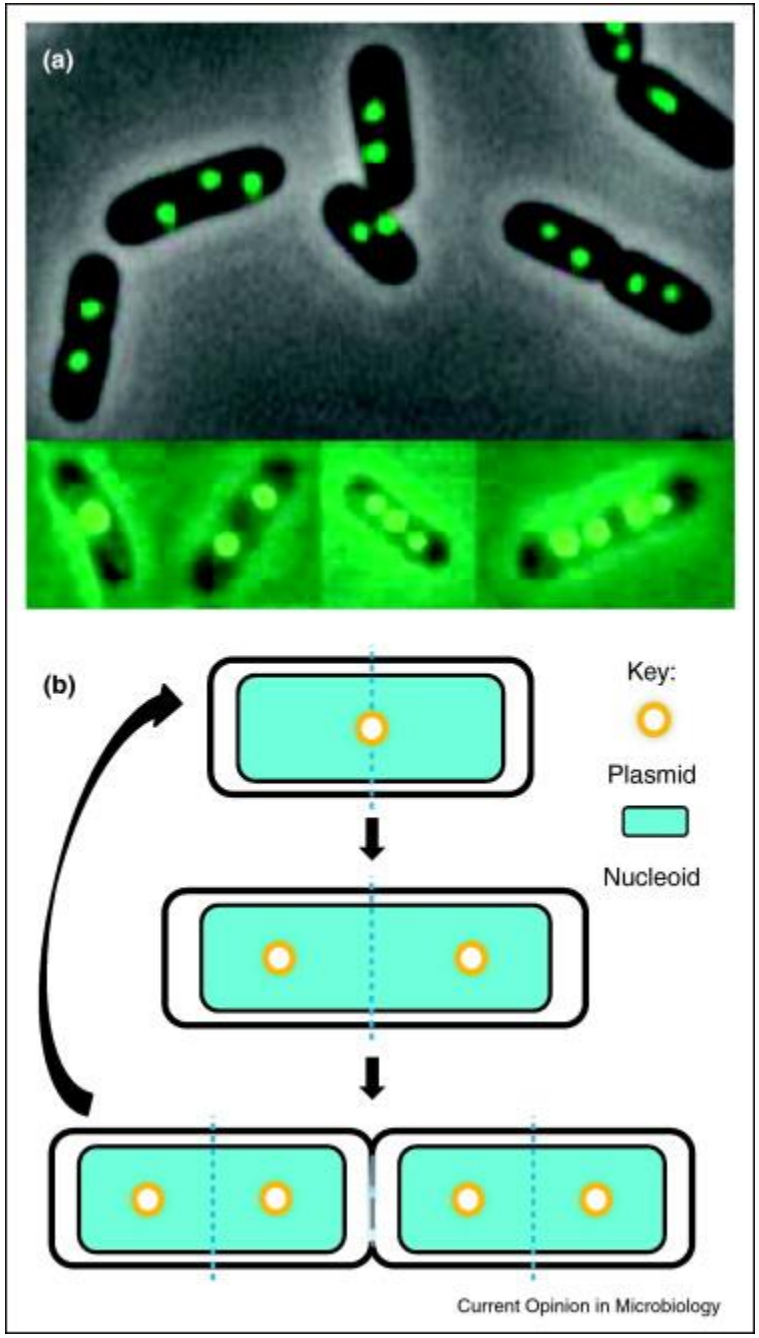
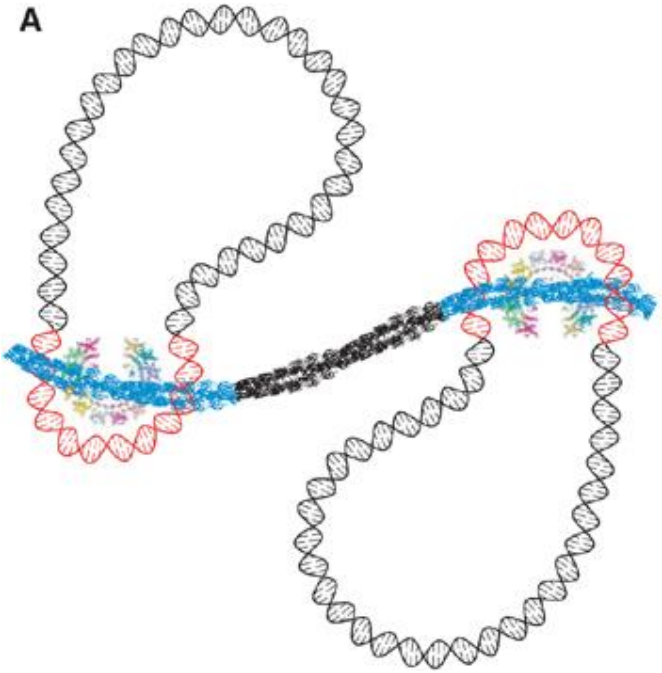


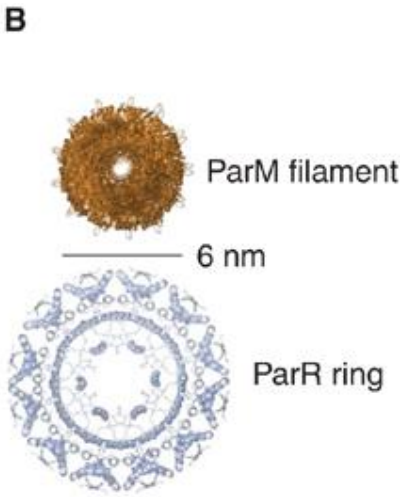
Diagram of the replication control region for IncI1 plasmids. RepZ is the main replication initiation protein and interacts with the origin of replication (*ori*), which is near repZ, to initiate replication of the plasmid sequence. Termination of plasmid replication occurs at *CIS*, which is located between *repZ* and *ori* (57). (B) Predicted RNA structure of the replication control (Rep) region of the IncI1 plasmid and predicted mechanisms of replication control. Control of *repZ* translation, and subsequently control of plasmid replication and copy number, is associated with the negative regulator *inc* and the positive regulator *repY*. To control replication, *inc* mRNA binds to the *inc* sequence and blocks the ribosomal binding site to inhibit RepY translation. To activate replication, *inc* mRNA is unbound from *inc*, allowing translation of RepY, which facilitates pseudoknot formation (binding of structure I to structure III at the binding sites indicated in red) that opens the ribosomal binding site to facilitate RepZ expression (based on data from reference 55).

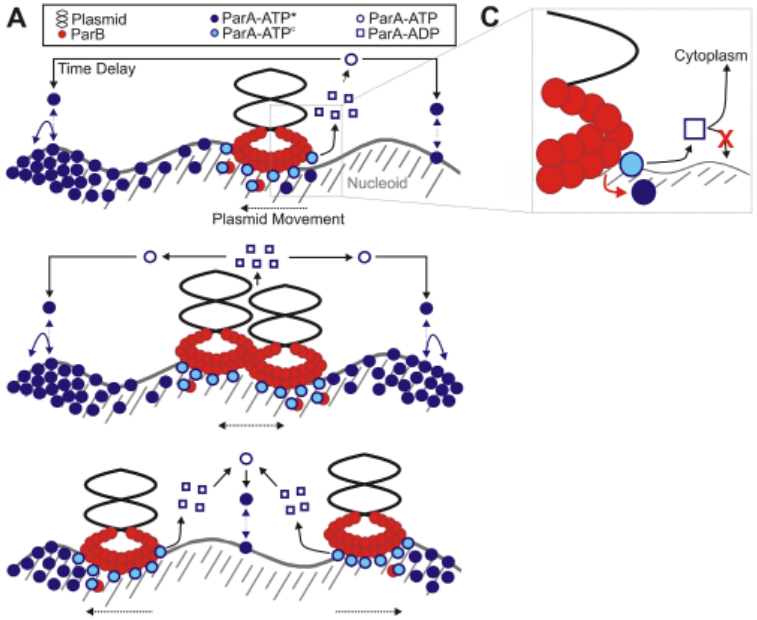


Plasmids control their segregation in the daughter cells

Szardenings F et al., 2011. Regular distribution of plasmids on the bacterial nucleoid confers genetic stabilisation of plasmids by type I *par* loci. Current Opinion in Microbiology 14 (6): 712-718







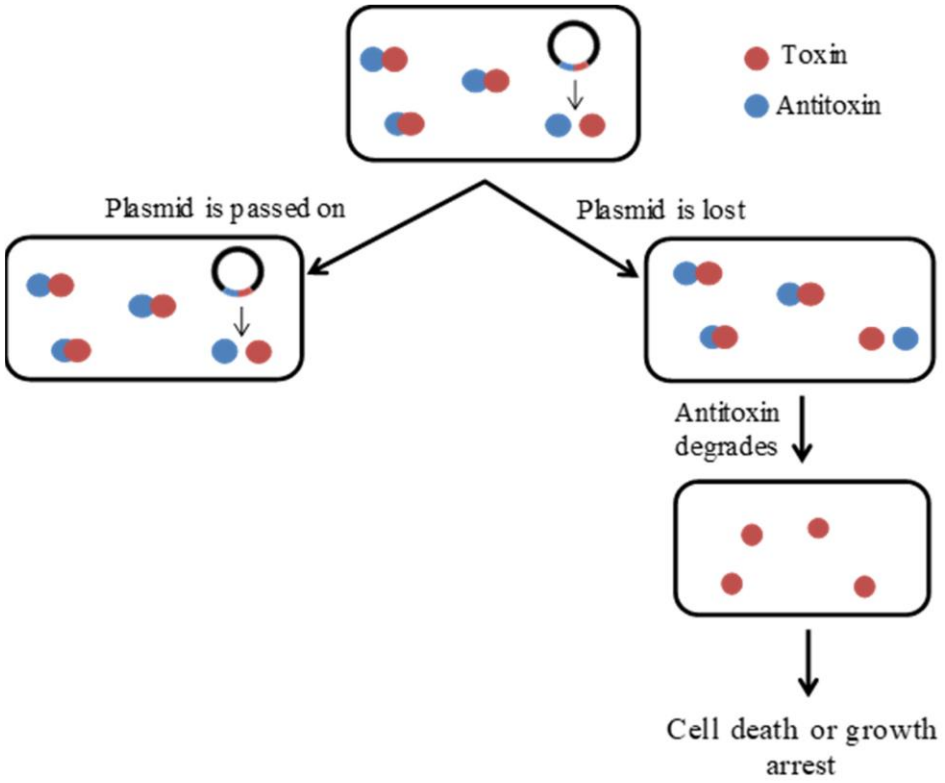
Model of R1 plasmid segregation.

Structural analysis of the ParR/*parC* plasmid partition complex J Møller-Jensen, S Ringgaard, CP Mercogliano, K Gerdes and J Löwe *The EMBO Journal* (2007) **26,** 4413–4422

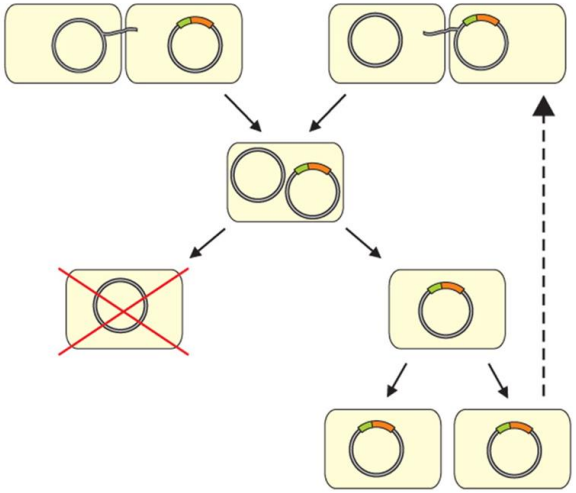
Toxin-antitoxin (TA) loci encode two-component systems that consist of a stable toxin and an unstable antitoxin

The role of TA systems in the plasmids: FUNCTION

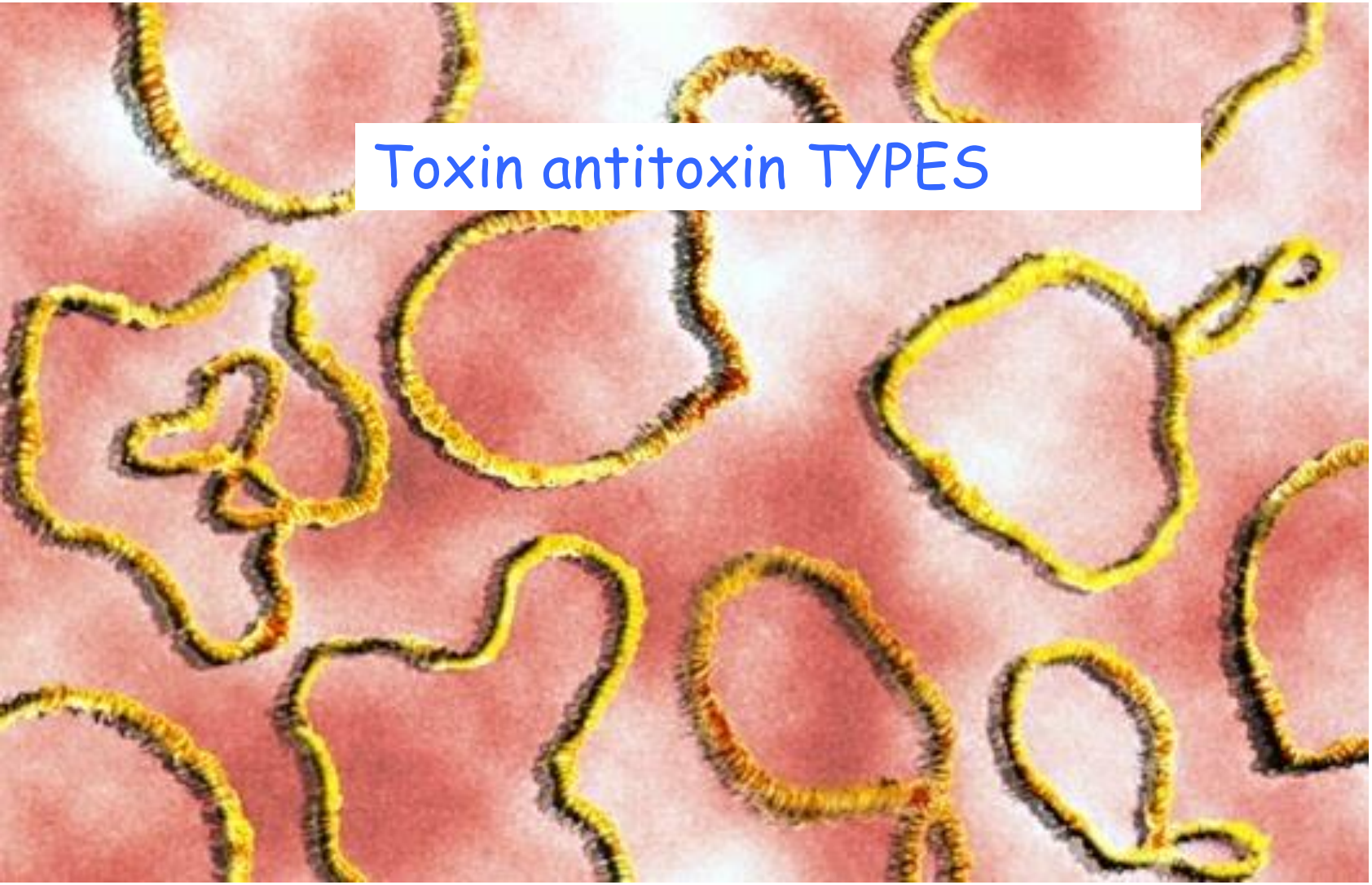
TA systems on plasmids confer stability of maintenance through **post-segregational killing (PSK)**



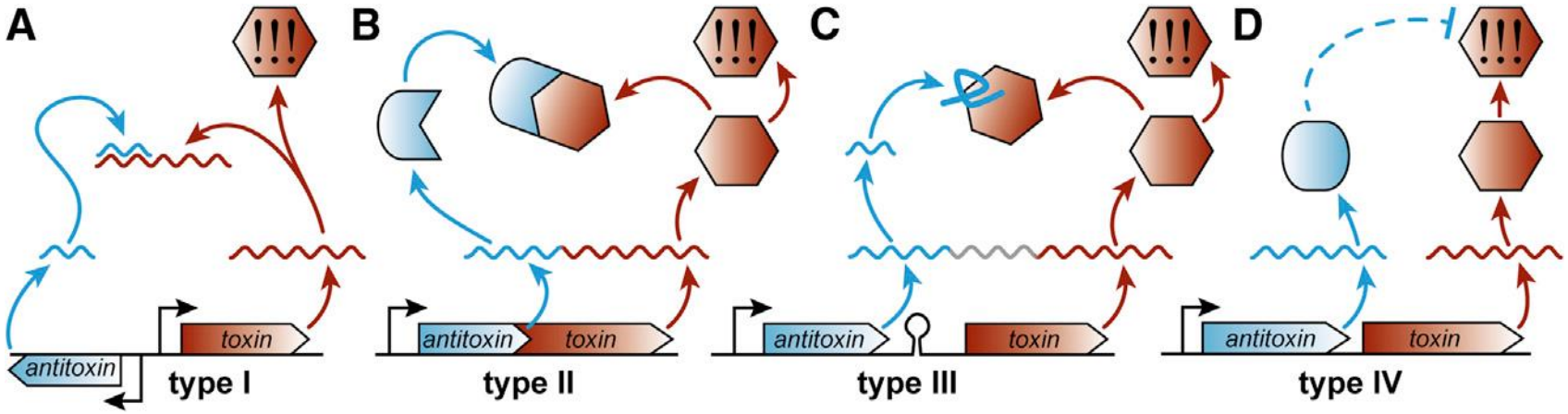
Exclusion of co-existent incompatible plasmids



Simon J et al. (2013) Toxin–antitoxin systems, Mobile Genetic Elements, 3:5, e26219
Kamruzzaman M, Wu AY, Iredell JR. Biological Functions of Type II Toxin-Antitoxin Systems in Bacteria. Microorganisms. 2021;9(6):1276


Toxin antitoxin TYPES

Classified in VI types by the nature and activity of the antitoxin



antitoxin RNA interacts with toxin RNA

antitoxin protein interacts with toxin protein

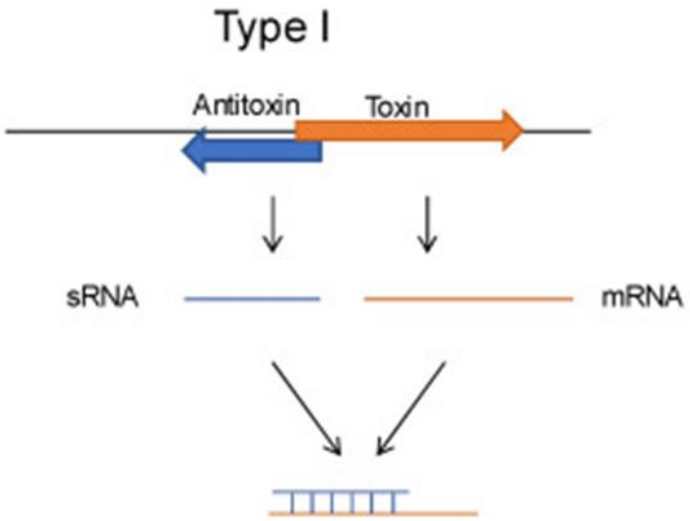
antitoxin RNA interacts with toxin protein

antitoxin protein interacts with other proteins modified by the toxin

Harms A, Brodersen DE, Mitarai N, Gerdes K. Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology. Mol Cell. 2018 7;70(5):768-784.

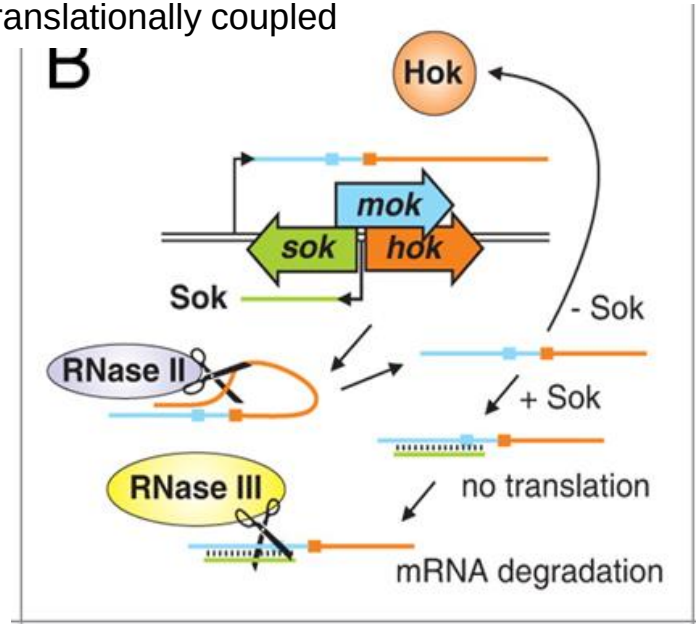
Type I: the antitoxin is a small antisense RNA complementary with the toxin encoding mRNA

Both Gram-negative and Gram-positive bacteria
Type I toxins are small hydrophobic proteins (less than 60 aa) containing a potential transmembrane domain, inducing pores into cell membranes



hok/Sok
bsrG/SR4
ldr/Rdl
tisB/IstR1
ibs/Sib
shoB/OhsC
symE/SymR

hok and mok are translationally coupled



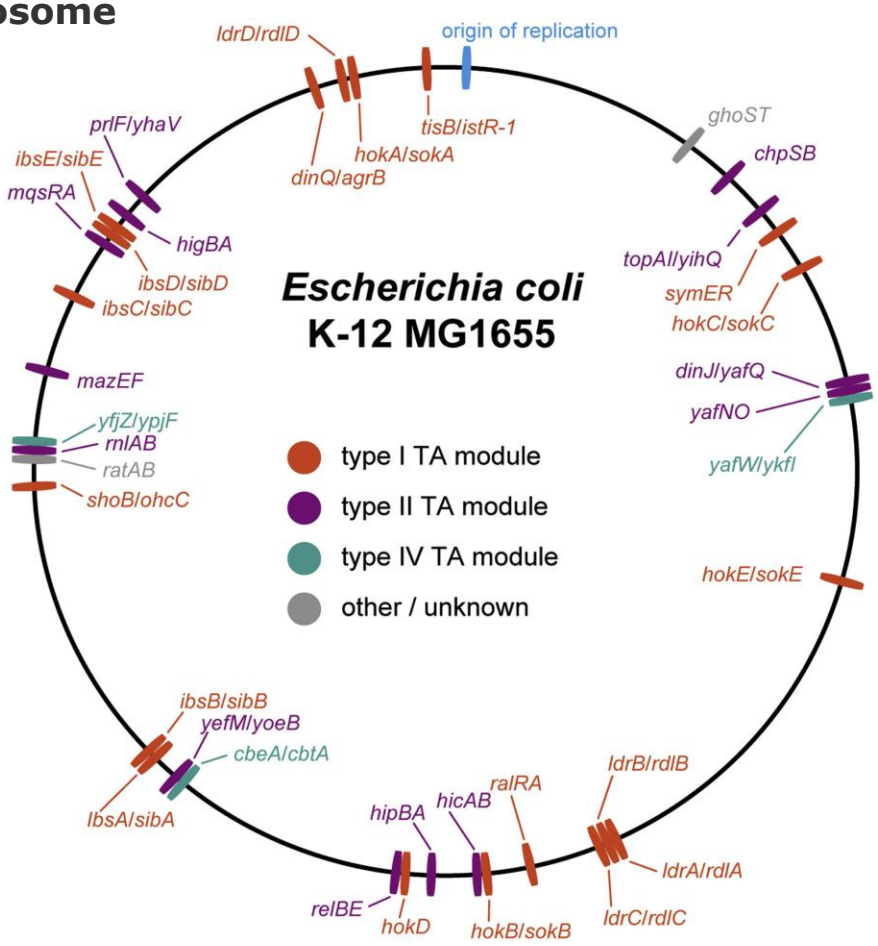
Simon J et al. (2013) Toxin–antitoxin systems, Mobile Genetic Elements, 3:5, e26219

Brantl S. Bacterial type I toxin-antitoxin systems. RNA Biol. 2012 Dec;9(12):1488-90

Kamruzzaman M, Wu AY, Iredell JR. Biological Functions of Type II Toxin-Antitoxin Systems in Bacteria. Microorganisms. 2021;9(6):1276.

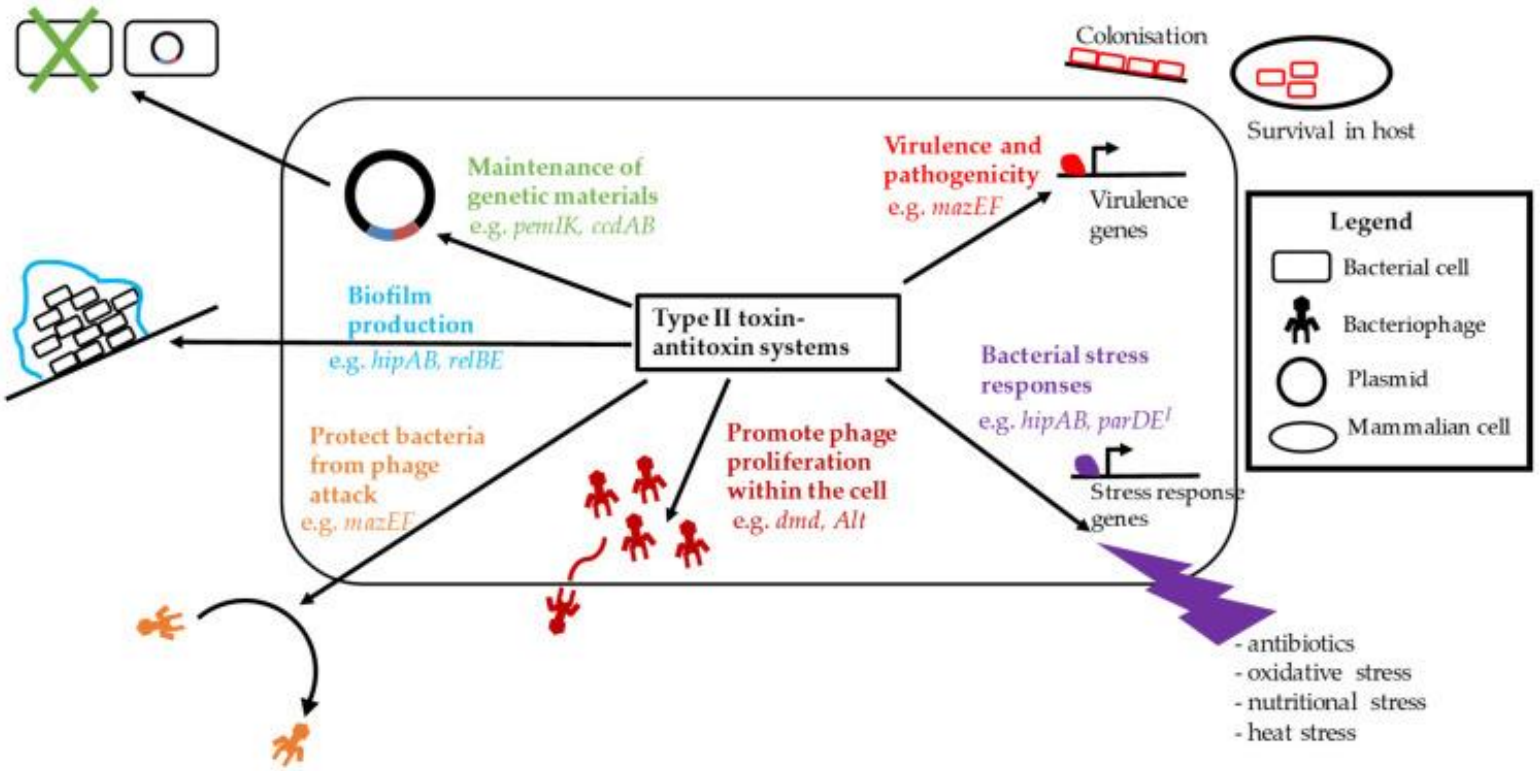

Toxin antitoxin: not only on plasmids

TA systems in *Escherichia coli* chromosome



Harms A, Brodersen DE, Mitarai N, Gerdes K. Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology. Mol Cell. 2018 7;70(5):768-784.

The role of TA systems



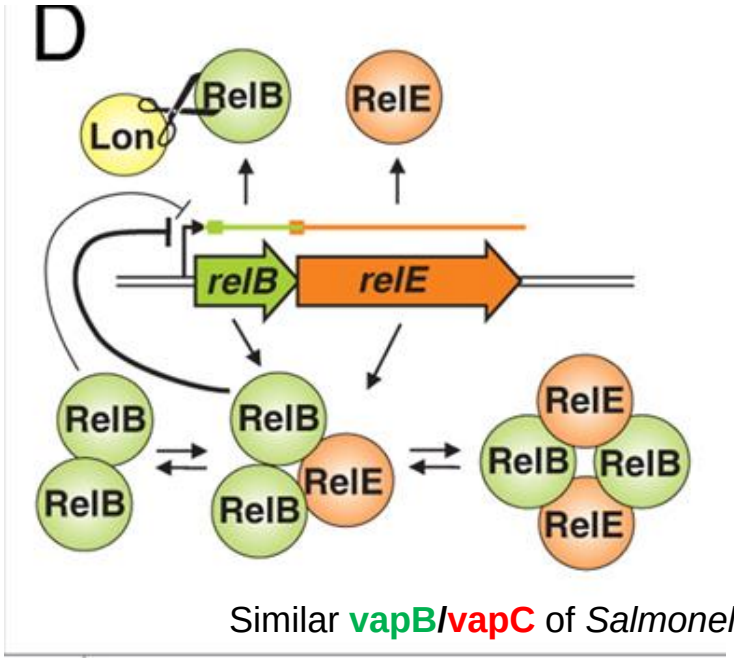
Kamruzzaman M, Wu AY, Iredell JR. Biological Functions of Type II Toxin-Antitoxin Systems in Bacteria. *Microorganisms*. 2021;9(6):1276

Type II: the antitoxin is a protein that interacts post-translationally with the toxin protein

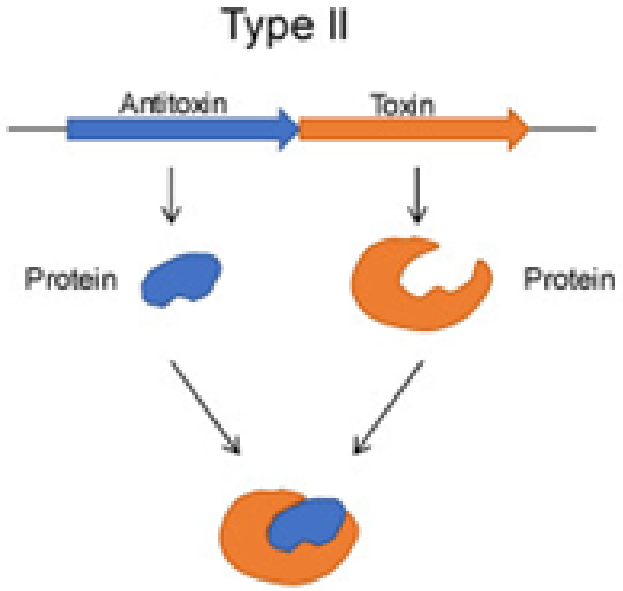
The *relB*/*relE* system from *E. coli*

2:1 complex RelB2 RelE inhibits the promoter

2:2 complex RelB2 RelE2 cannot bind the promoter transcription is activated

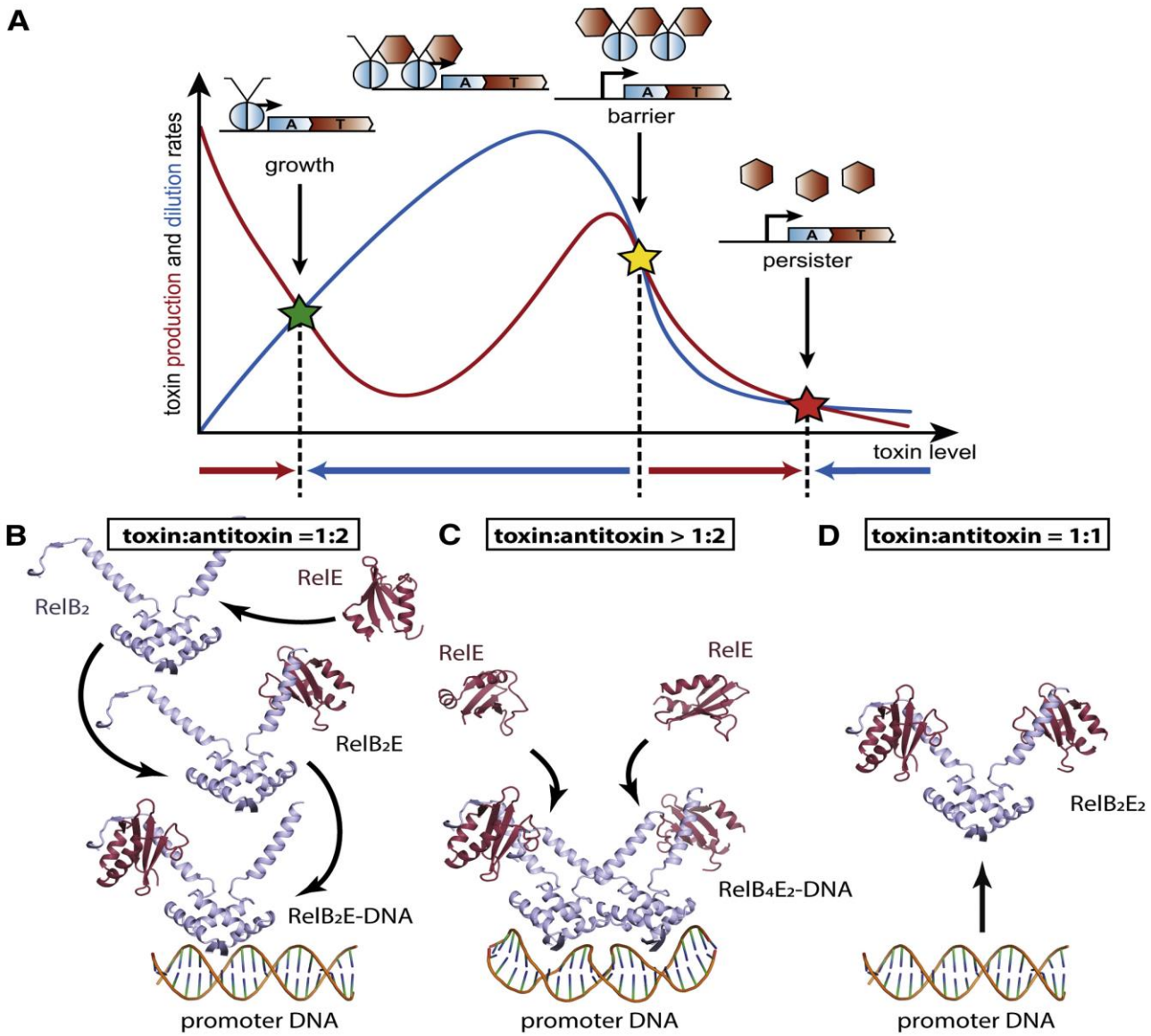


Similar **vapB/vapC** of *Salmonella*



Simon J et al. (2013) Toxin–antitoxin systems, Mobile Genetic Elements, 3:5, e26219

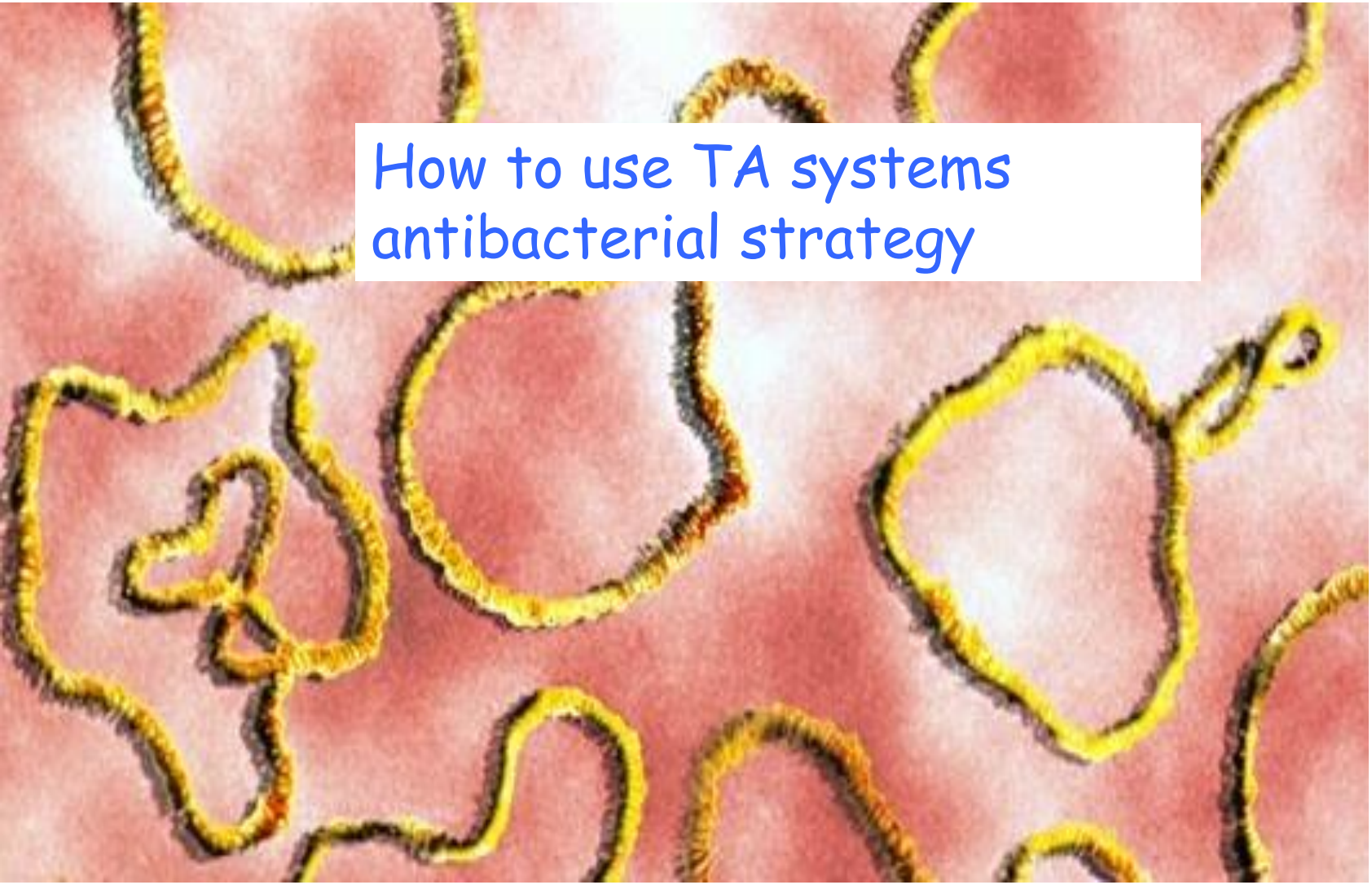
Kamruzzaman M, Wu AY, Iredell JR. Biological Functions of Type II Toxin-Antitoxin Systems in Bacteria. Microorganisms. 2021;9(6):1276.

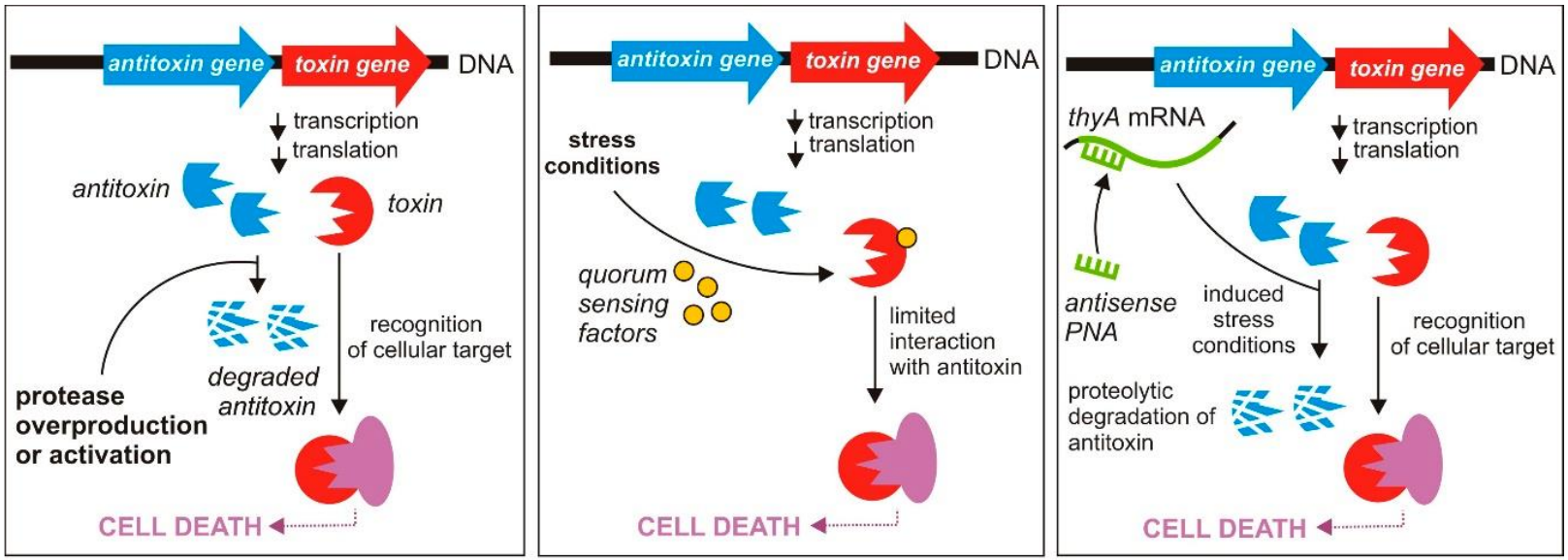


Harms A, Brodersen DE, Mitarai N, Gerdes K. Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology. Mol Cell. 2018 7;70(5):768-784.

TADB 2.0: Y. Xie, Y. Wei, Y. Shen, X. Li, H. Zhou, C. Tai, Z. Deng and H.Y. Ou (2018) TADB 2.0: an updated database of bacterial type II toxin-antitoxin loci. *Nucleic Acids Research,* 2018, 46:D749-D753.

TADB provides an web-interface, allowing users to view an entire genome's TA loci repertoire within the context of the whole replicon and to access individual pages dedicated to each TA locus pair, toxin and antitoxin as required


How to use TA systems
antibacterial strategy



Proposed antibacterial strategies based on the indirect activation of toxins of TA systems:

(a) activation of the Lon or ClpP proteases that degrade antitoxins (with a plasmid carrying a cloned protease gene);

(b) triggering TA systems by quorum sensing factors (mazEF/pentapeptide extracellular death factor EDF) [Kumar and Engelberg-Kulka, 2014]

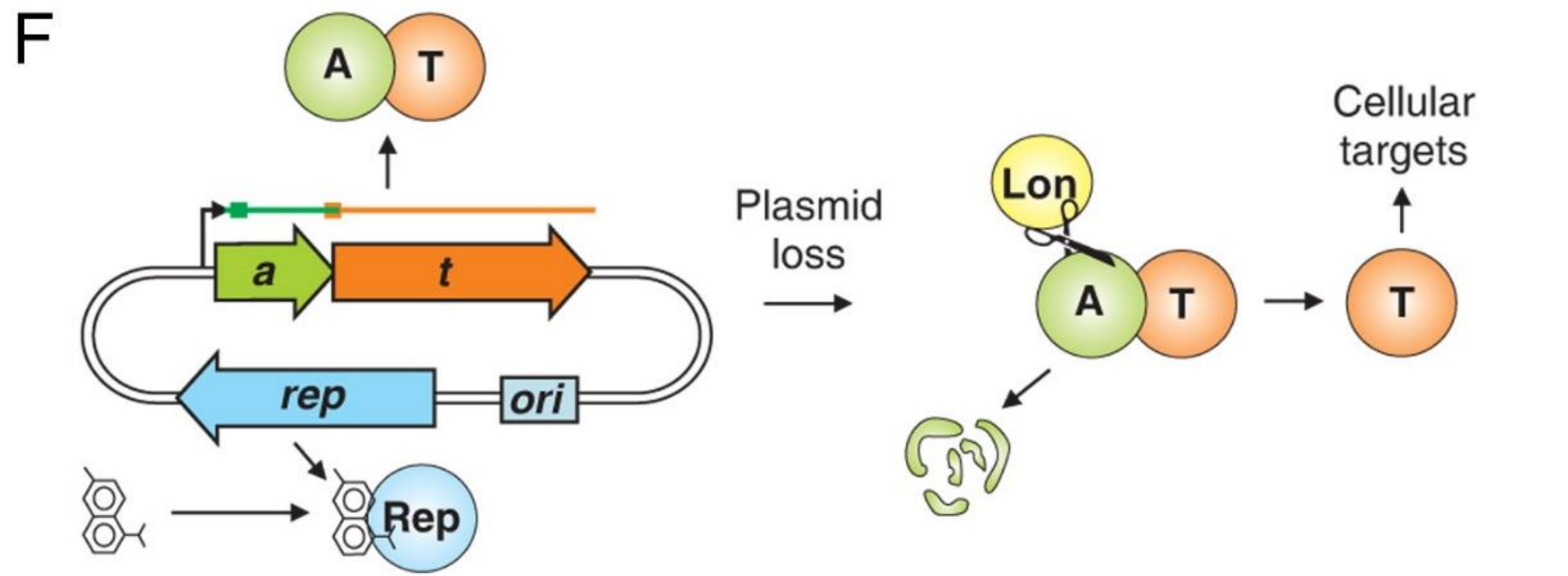
(c) triggering TAs by artificial induction of the stringent response sequence-specific PNAs targeting the *thyA* gene of E. coli, to trigger MazF toxin production by inducing thymine starvation [Równicki, et al.,2018]

Równicki, M et al., (2020). Targeting Type II Toxin-Antitoxin Systems as Antibacterial Strategies. *Toxins*, *12*(9), 568

How can we use TA systems on plasmids against bacteria?

TA systems can be used to design antibacterial drugs

Plasmids can be cured, and cured cells can be killed off by stable toxins from plasmid-mediated TA systems



Simon J et al. (2013) Toxin–antitoxin systems, Mobile Genetic Elements, 3:5, e26219

nature biotechnology

Letter | Published: 15 April 2019

Engineered toxin–intein antimicrobials can selectively target and kill antibiotic-resistant bacteria in mixed populations

Rocío López-Igual, Joaquín Bernal-Bayard, Alfonso Rodríguez-Patón, Jean-Marc Ghigo & Didier Mazel ✉

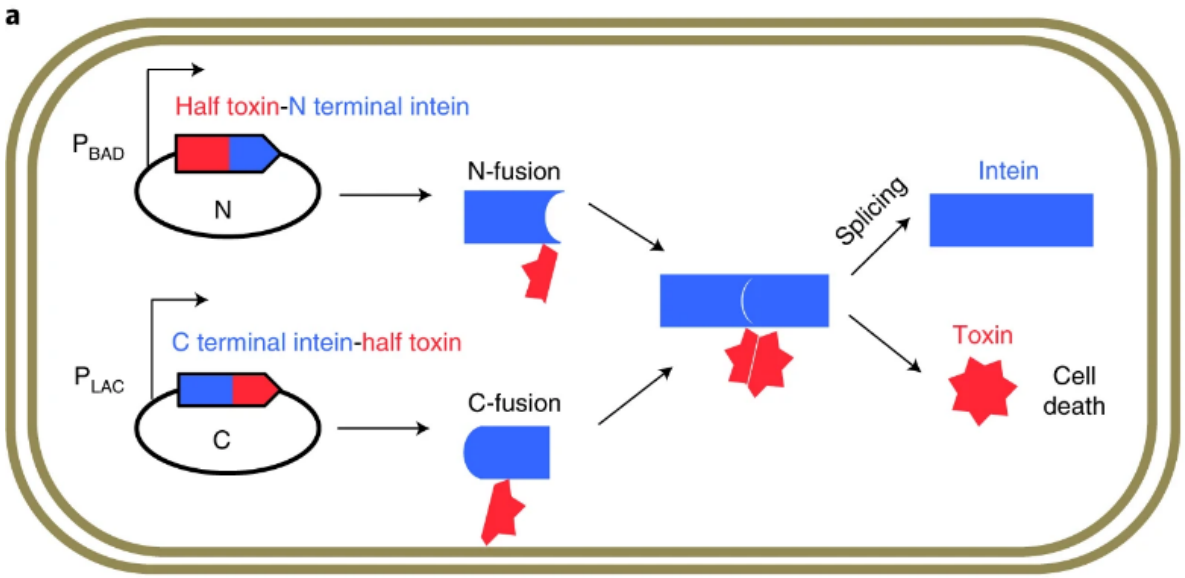
Nature Biotechnology **37**, 755–760 (2019) | Cite this article

V. cholerae causes between 21,000 and 143,000 deaths from cholera per year[11]. The most recent cholera pandemics involved the O1 and O139 serogroups.

Virulence in *V. cholerae* is coordinated by the master transcriptional activator ToxR, which regulates the ToxR regulon[12], and includes the **cholera toxin genes**.

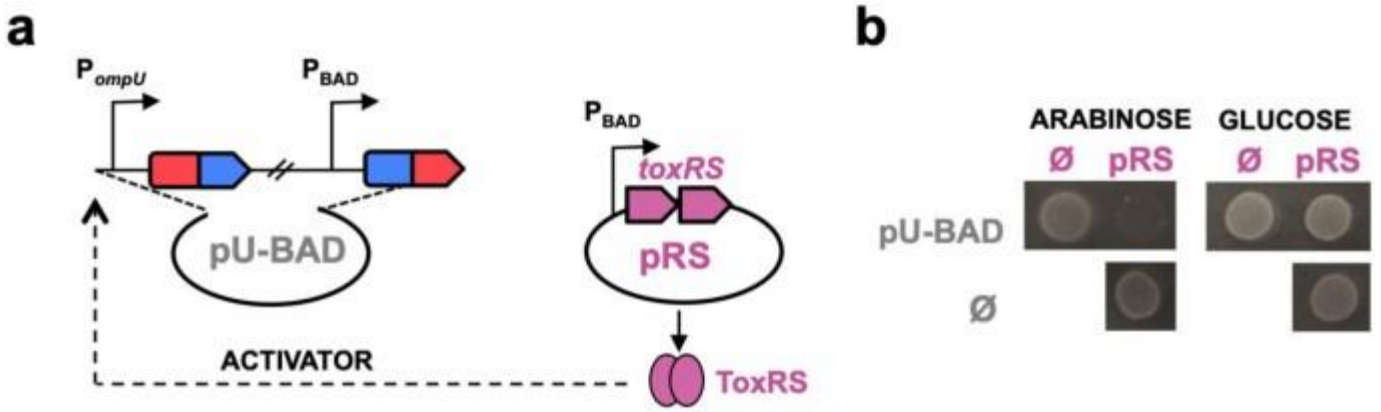
Cholera epidemics are associated with **antibiotic resistance due to resistant genes present on an integrative and conjugative element named SXT** (from sulfamethoxazole and trimethoprim resistance). SXT can carry genes that confer resistance to sulfamethoxazole (*sul*2), trimethoprim (*dfrA*1 and *dfr*18), streptomycin (*strB*), chloramphenicol (*floR*) and tetracycline (*tetA*) and was first described in *V. cholerae* serogroup O139 (ref. [13]).
SXT also encodes functions promoting its excision, dissemination by conjugation and integration, as well as the transcription factors that control expression of these functions[13].

Our previous experience with type II toxins[14,15] taught us that basal expression of a full-length toxin gene from P_{BAD} is sufficient to kill the *E. coli* host.
To avoid this, we designed a genetic module containing a toxin split by an intein, and in our module the split toxin–intein can be activated only by ToxR.
Inteins are protein sequences embedded into a host protein (extein) from which they are autocatalytically excised in a process called protein splicing.
During protein splicing, the intein ligates the extein extremities and allows the reconstitution of the mature protein. In nature, a few examples of split inteins also exist allowing the assembly of a single protein from two genes[16].
We split the type II toxin gene *ccdB* (Plasmid pToxInt) into two parts, each of which is associated with half of a split intein. Split inteins have been used in several biotechnological tools[17] and enable control of toxic protein functions in vivo[18]. We used the split-intein DnaE, which is present in the *dnaE* gene of *Nostoc punctiforme*. DnaE is well characterized and has a high rate of trans-splicing[19]. Using inteins enables strict control of toxin production and avoids toxicity due to basal expression[14,15] (

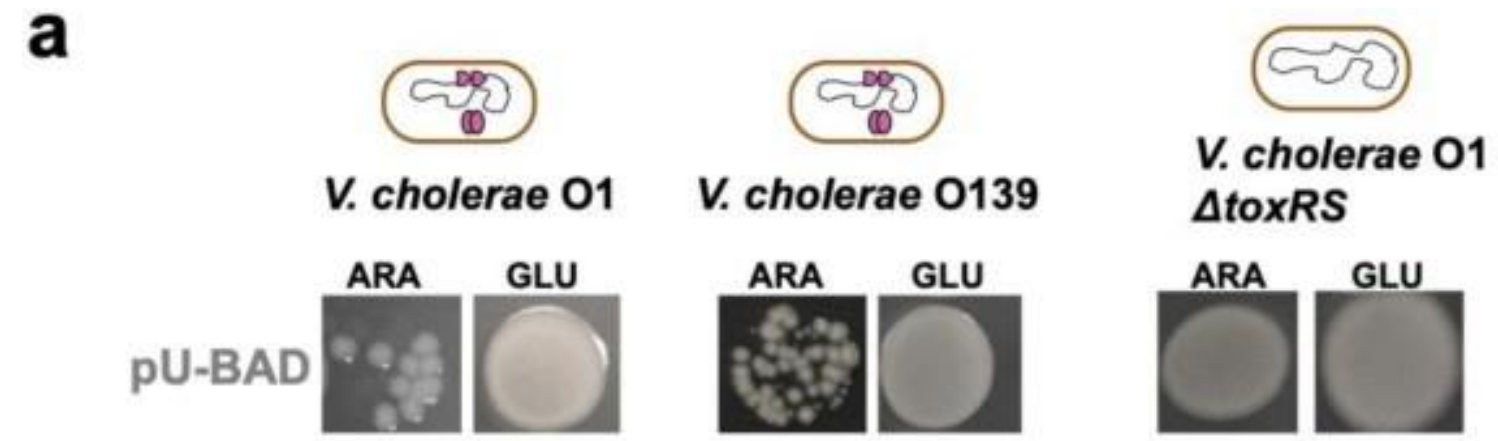


The type II toxin gene *ccdB* is cloned into two parts, each of which is associated with half of a split intein.

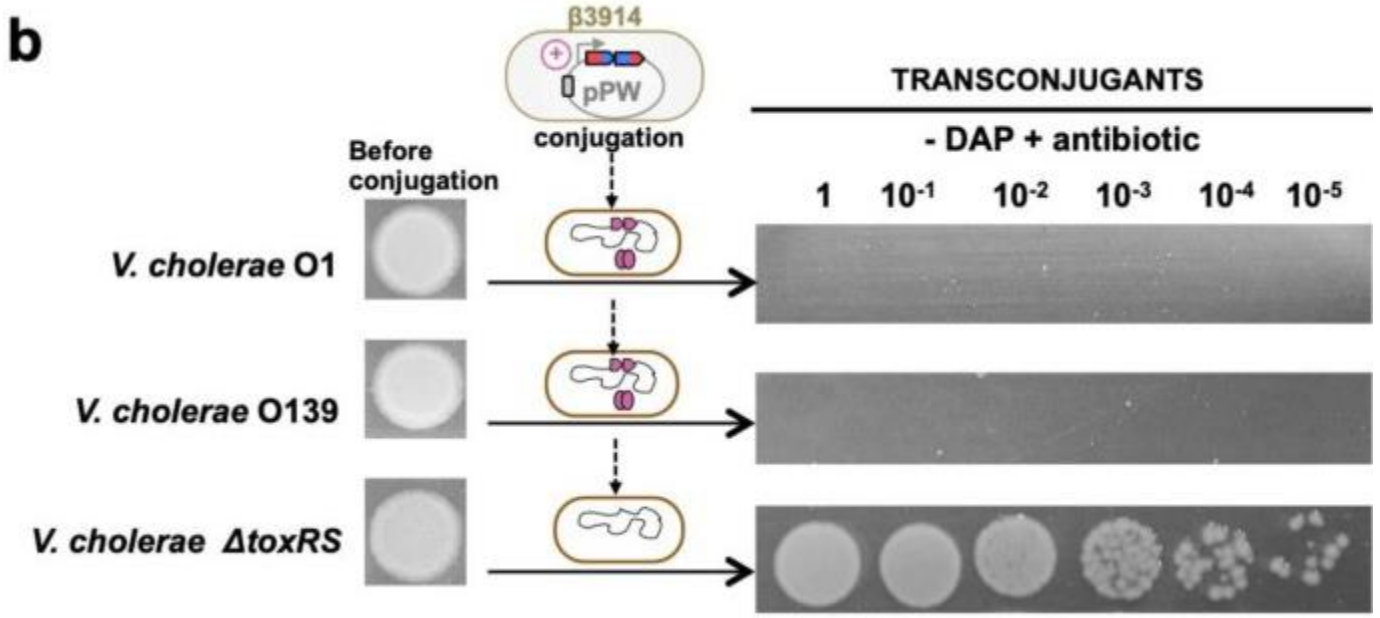
In *V. cholerae* one of the ToxRS-regulated genes encodes a membrane porin, OmpU[21]. **We cloned the N fusion of CcdB-intein downstream of the *ompU* promoter** (regulated by ToxRS) and the **C fusion under P_{BAD} in the same plasmid (pU-BAD).** The functionality of pU-BAD was tested in an *E. coli* DH5α strain expressing the *V. cholerae toxRS* operon from a second plasmid (pRS). On arabinose-mediated induction of *toxRS* expression, only bacteria containing both pU-BAD and pRS plasmids died

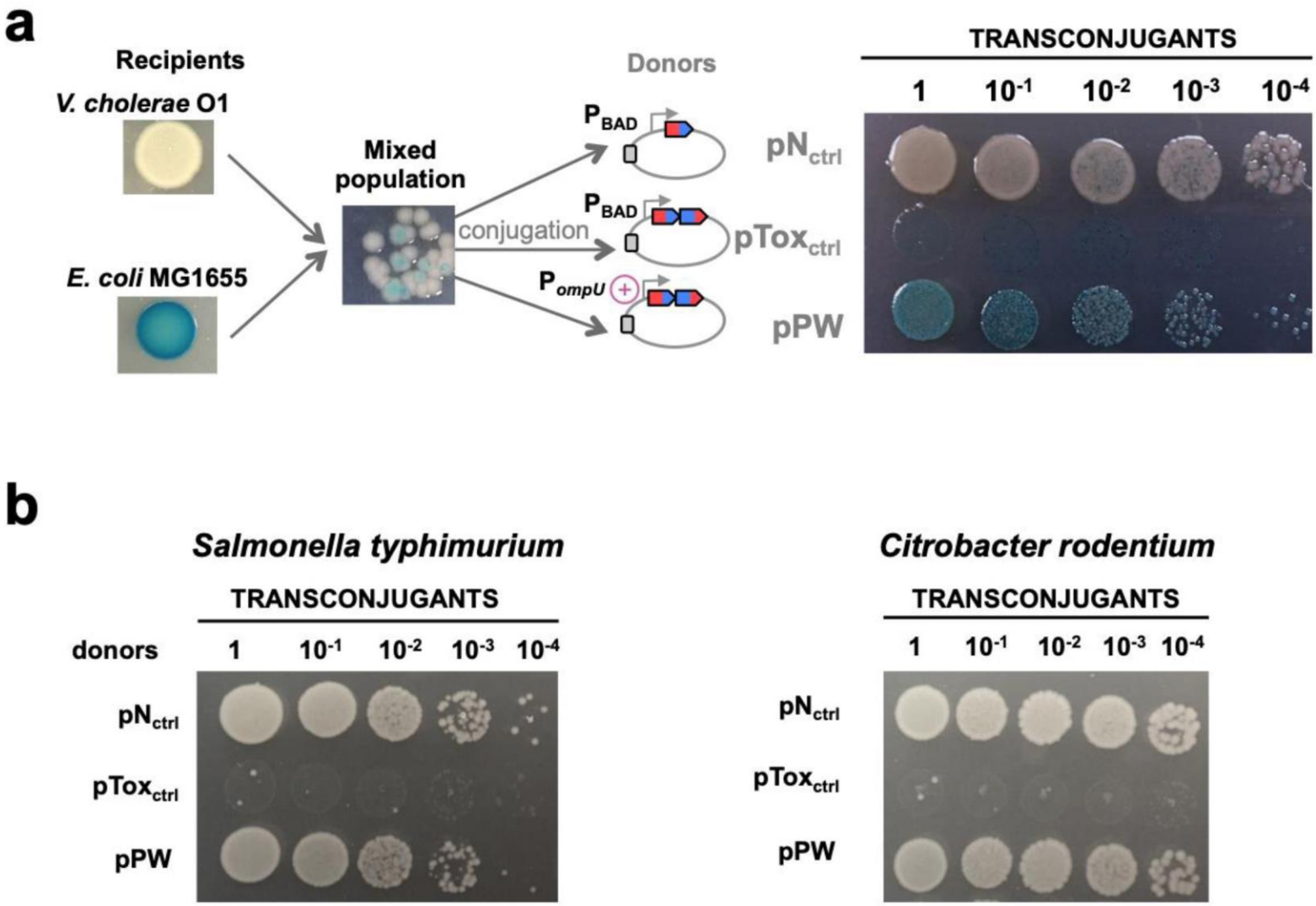


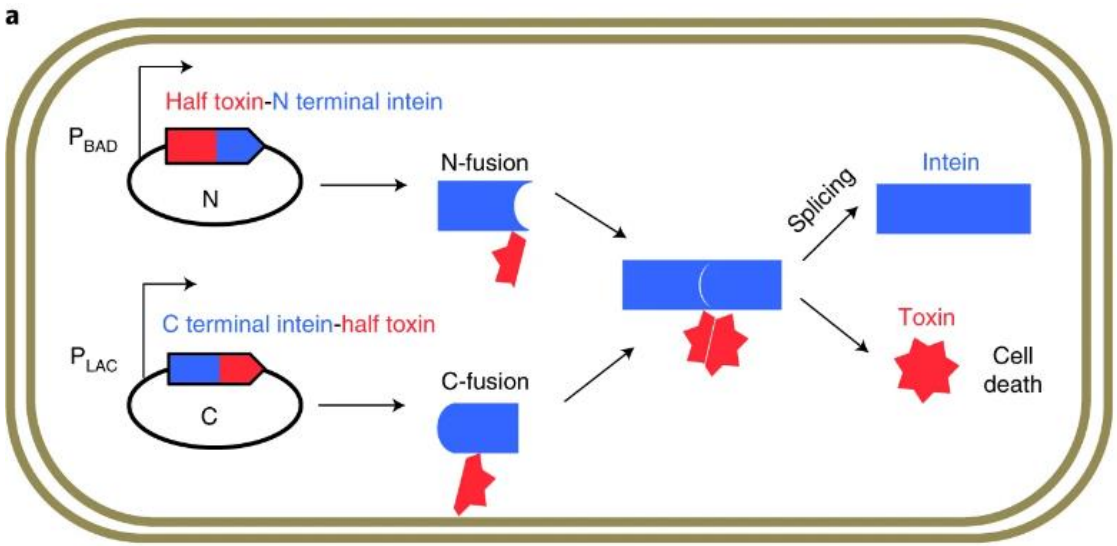
Test of pU-BAD and of pPW, the genetic pathogenic-weapon, in *V. cholerae* serogroups O1 and O139.

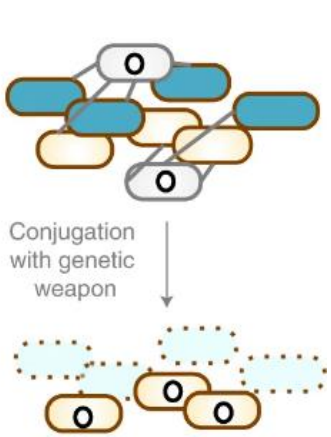


we cloned a split-toxin–intein operon under the control of *ompU* promoter in a plasmid and added an origin of transfer (*oriT*) to render it conjugative (plasmid pPW). Conjugation is carried out from donor strain *E. coli* β3914, an MG1655 *ΔdapA* that contains the RP4 conjugative machinery integrated into its chromosome.
pPW was introduced by conjugation into *V. cholerae* strains O1, O139 and an O1-*ΔtoxRS* mutant, but only the *ΔtoxRS* strain was able to grow after transfer of the pPW plasmid, demonstrating that it kills only *Vibrio* expressing ToxR.



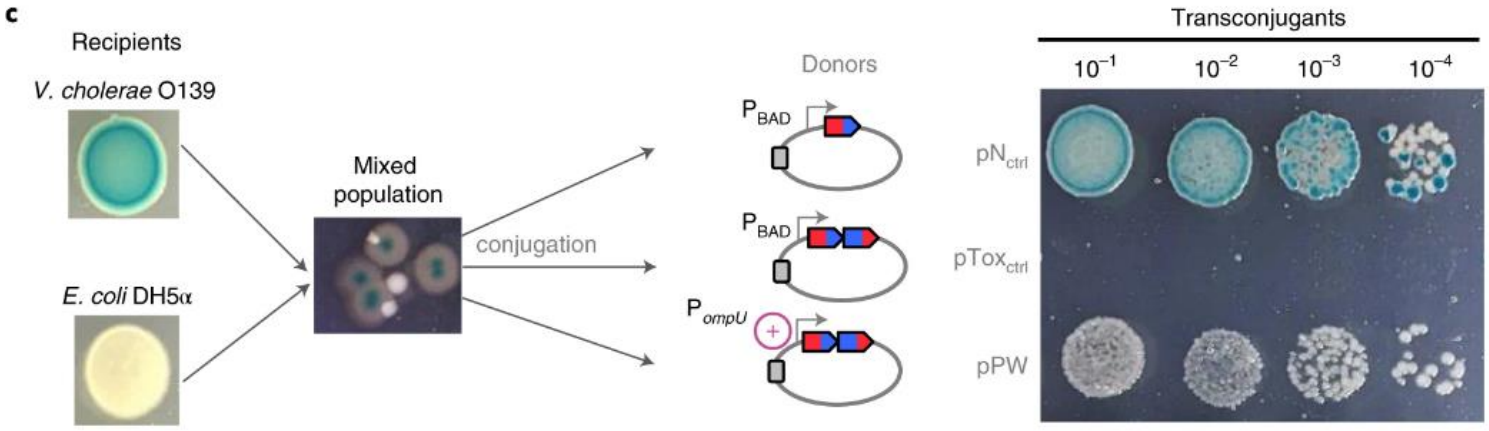

a
Recipients
V. cholerae O1
E. coli MG1655
Mixed population
conjugation
Donors
P BAD
P BAD
P ompU
pN ctrl
pTox ctrl
pPW
TRANSCONJUGANTS
1
10-1
10-2
10-3
10-4
b
Salmonella typhimurium
TRANSCONJUGANTS
donors
1
10-1
10-2
10-3
10-4
pN ctrl
pTox ctrl
pPW
Citrobacter rodentium
TRANSCONJUGANTS
1
10-1
10-2
10-3
10-4
pN ctrl
pTox ctrl
pPW





The type II toxin gene *ccdB* is cloned into two parts, each of which is associated with half of a split intein.

The *ccdB*/intein are cloned under the control of ToxR that regulates the cholerae toxin genes

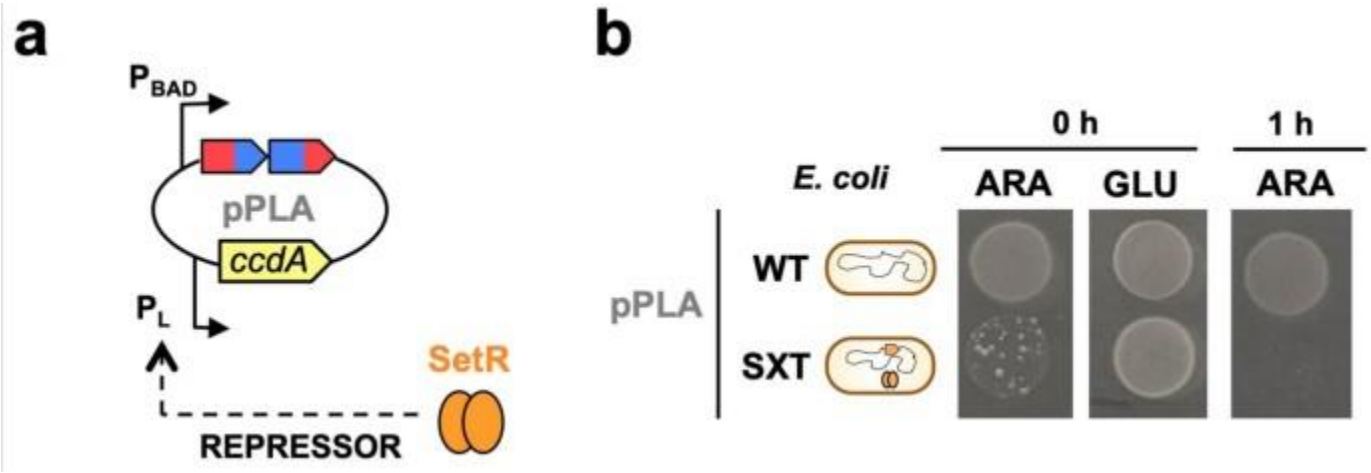


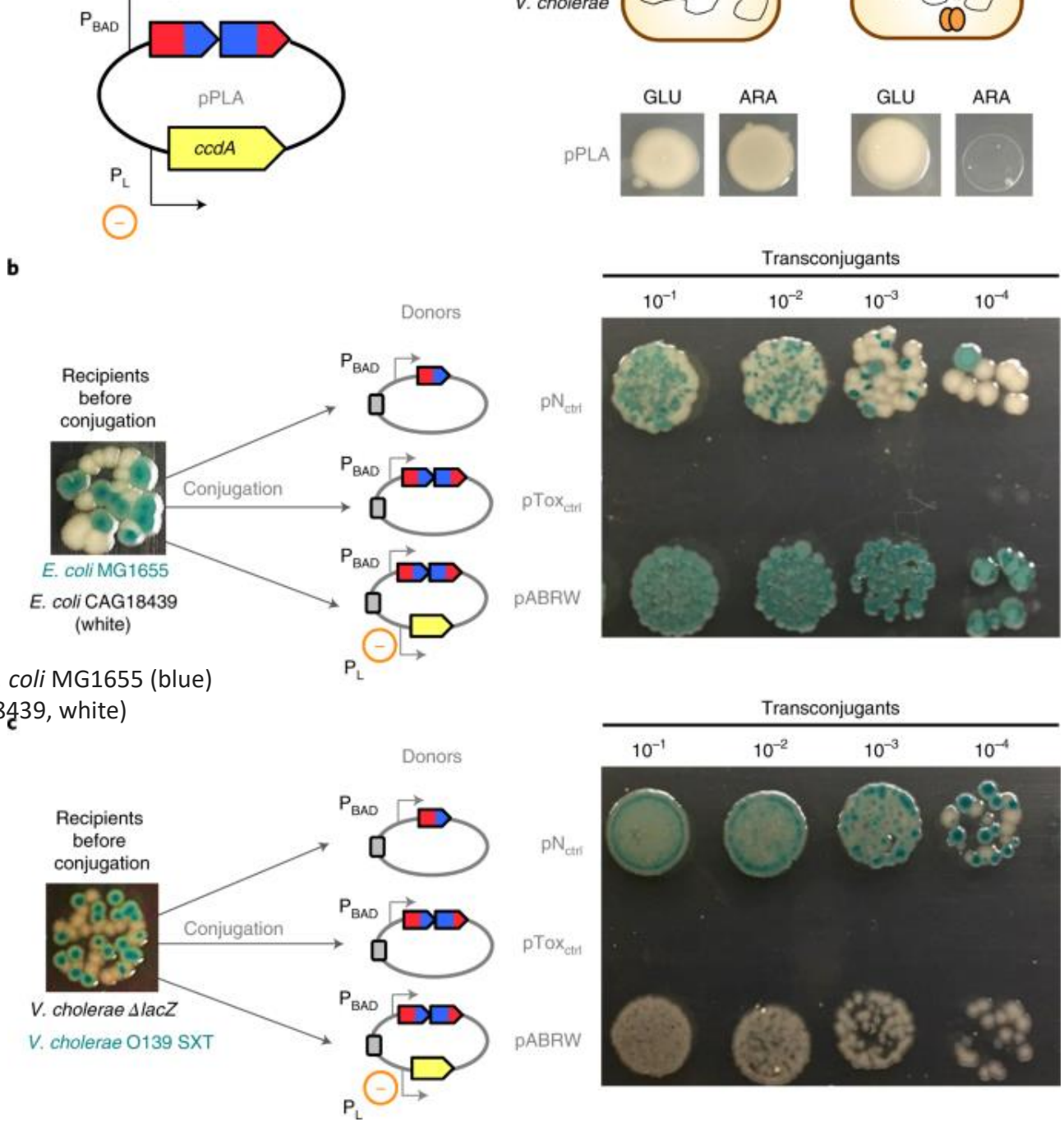
ToxR

a split-intein toxin could kill ABR bacteria present in a community.

The SXT integrative and conjugative element family in *V. cholerae* includes various antibiotic resistance genes[13]. The SXT chassis encodes several transcription factors that regulate SXT transmission including the **SetR repressor**[13].

We designed a module to detect SXT carriage and kill SXT-harboring bacteria by implementing an additional component into our antimicrobial: the *ccdA* gene, which encodes the antitoxin partner of CcdB. ***ccdA* was cloned downstream of the SXT PL promoter**, which is controlled by the SetR repressor, in a plasmid also containing the *ccdB*-intein operon regulated by the P_{BAD} promoter (pPLA plasmid).



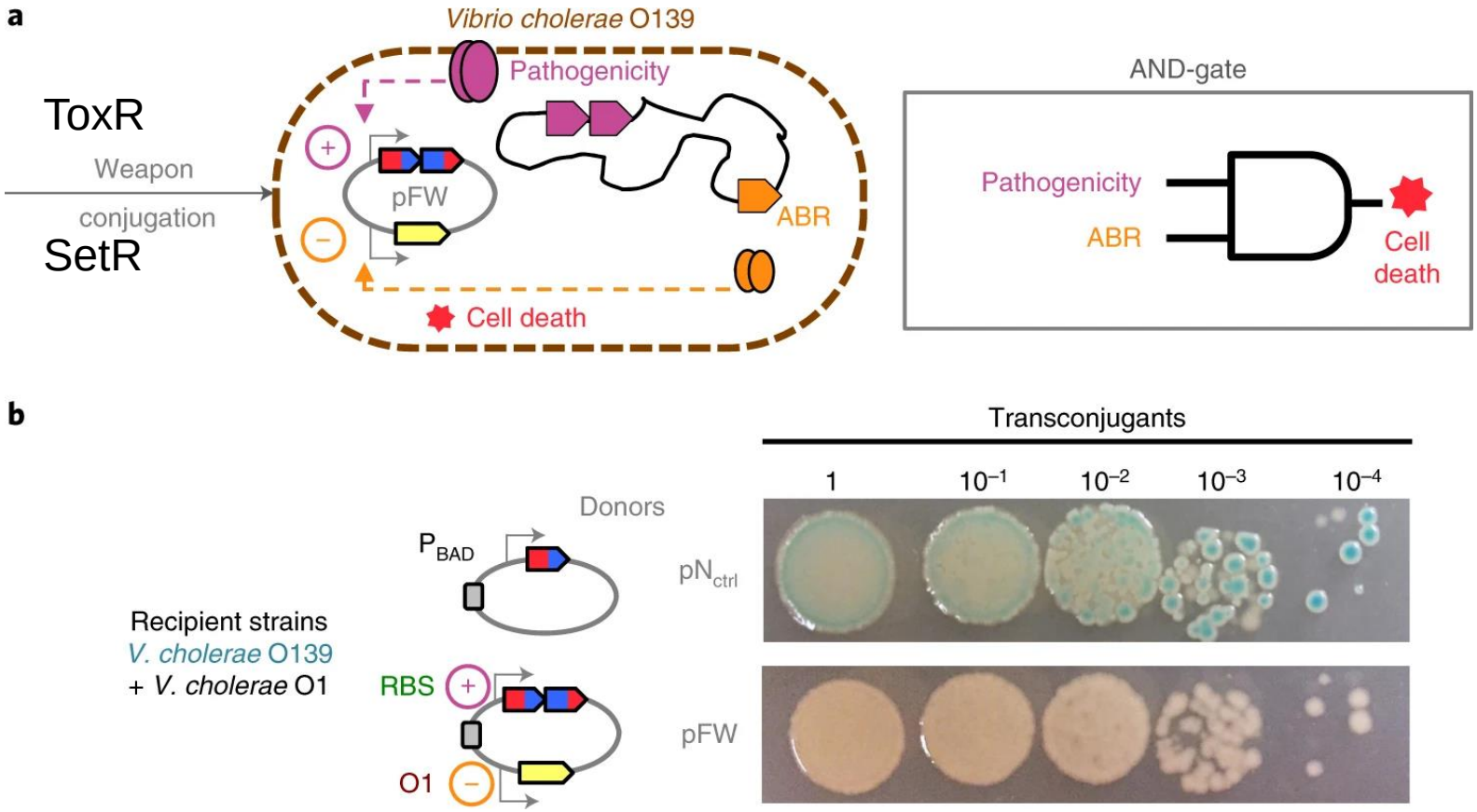


Mixed population of *E. coli* MG1655 (blue) and *E. coli* SXT (CAG 18439, white)

Mixed population of *V. cholerae* O139-SXT (blue) and *V. cholerae* O1 Δ*lacZ* (white) as recipients for conjugation using β3914 as donor strain containing pN_{ctrl}, $pTox_{ctrl}$ or pABRW plasmids,

Rocío López-Igual, Joaquin Bernal-Bayard, Alfonso Rodríguez-Patón, Jean-Marc Ghigo, Didier Mazel. Engineered toxin–intein antimicrobials can selectively target and kill antibiotic-resistant bacteria in mixed populations. *Nature Biotechnology*, 2019, 37 (7), pp.755-760.

The antitoxin ccdA under the negative control of SetR that is produced by SXT integrative element in AMR *V. cholerae*



Schematic representation of the specific killing of *V. cholerae* O139 after pFW conjugation (left). Schematic display of the corresponding AND-logic gate (right). **b**, Conjugation from β3914 of either pN_{ctrl} or pFW, of *V. cholerae* serogroup O139 (blue) and O1 (white) as a recipient mixed population. Transconjugants were selected on Mueller–Hinton + Sp (plasmid marker). The pFW plasmid was obtained after a change in a ribosomal binding site (RBS) sequence of *ompU* promoter to increase translation of toxin–intein fusion and substitution of the O4 operator sequence by O1 operator sequence (see Methods) to increase SetR binding affinity to the PL promoter and consequently increase repression. Only the *V. cholerae* serogroup O1, which is devoid of SXT in its genome, was detected after pFW conjugation, demonstrating the specific killing of serogroup O139, which contains both chromosomally encoded ToxR and SetR, the chosen indicators of pathogenicity and antibiotic resistance, respectively. Pictures are representative of three independent experiments.