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The dualistic nature of integrative and conjugative elements

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List of Abbreviations: ICE, integrative and conjugative element; Mb, mega base pair; MGE, mobile genetic element; *oriC*, origin of replication of the chromosome; *oriT*, origin of transfer of a conjugative element; PSK, post-segregational killing; T4SS, type IV secretion system.

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Integrative and conjugative elements (ICEs) are mobile genetic elements that play a key role in bacterial adaptation. Such elements are found in almost every bacterial genera and species, and often code for adaptive traits conferring selective advantages to their host. ICEs maintain by integrating into and replicating along with a replicon of the host genome. ICEs can propagate by conjugative transfer toward a recipient cell following excision from the replicon as a circular covalently-closed molecule. For a long time, the excised form of ICEs was assumed to be non-replicative. This assumption predicts that excised ICEs are sensitive to loss during cell division, unless they carry stabilization systems such as addiction modules or antibiotic resistance genes. Over the past few years, growing evidence have been presented that support conditional replication of the circular intermediate as an intrinsic feature of ICEs. We recently confirmed this feature in the large family of SXT/R391 ICEs, which thrive in several species of *Enterobacteriaceae* and *Vibrionaceae*. Furthermore, we demonstrated that SXT/R391 ICEs encode a functional plasmid-like type II partition system that enhances their stability, such systems being probably encoded by other ICEs. The lifecycle of ICEs is therefore much more complex than initially thought as many ICEs may use plasmid-like features to improve their stability and dissemination.

Eight decades ago, at the dawn of the antibiotic era, the discovery and use of antimicrobials strongly improved the well-being of people by allowing a drastic decrease in mortality and morbidity caused by bacterial infections. Since then, our (over)confidence regarding the efficiency of therapies against bacterial infections using antibiotic compounds was shaken by the immense potential of adaptation of bacteria. Notably, horizontal gene transfer of mobile genetic elements (MGEs) was shown to be a key driver of this adaptation, enabling bacteria to quickly acquire new adaptive traits including multiple antibiotic resistances or alternative metabolic functions.[1-4] Integrative and conjugative elements (ICEs) are undoubtedly major players in bacterial adaptation. These genomic islands encode and disseminate a large variety of adaptive traits that are often helpful to the host cell to overcome different stresses, colonize new niches and/or improve the pathogenicity of their host.[4-11] ICEs are autonomous MGEs that can transfer by conjugation toward a recipient cell in direct contact. The maintenance of ICEs relies mainly on their integration into and replication along with a replicon of their bacterial host in a prophage-like manner. Their conjugative transfer involves their excision as a circular intermediate molecule that is the substrate for conjugative transfer through a type IV secretion system (T4SS), as observed for conjugative plasmids. Beyond their own transfer, ICEs extend their impact on genome plasticity *via* the mobilization of non-autonomous elements that hijack part of the ICE-encoded transfer functions, or by *Hfr*-like transfer of chromosomal DNA fragments.[12-16] Although ICEs have been studied for several decades in many laboratories worldwide, their biology is far from being completely understood. Notably, the apparent complexity of their lifecycle, which involves 2 distinct forms present in a disproportionate ratio among the same bacterial population and each exhibiting specific characteristics, clouds

our understanding of the way these MGEs operate.

We recently conducted a study on the dynamics of SXT/R391 ICEs by mainly using R391, a kanamycin-resistant prototypical member of SXT/R391 ICEs that was originally isolated from a *Providencia rettgeri* isolate from South Africa.[17] SXT/R391 ICEs constitute a large family of ICEs that has been thoroughly studied over the last decades for their diversity and biology.[6,18,19] Such elements are responsible for multidrug resistance acquisition and dissemination among *Vibrio cholerae* isolates during the seventh pandemic of cholera and other bacteria worldwide.[20,21] SXT/R391 ICEs share a common set of conserved genes that ensure their basic functions and integrate into the 5′ end of the *prfC* gene in the chromosome of numerous *Enterobacteriaceae* and *Vibrionaceae* (**Table 1**).[7,18] We showed that R391 stability was improved in the presence of selective pressure exerted by the addition of kanamycin, as well as in the presence of the ICE-encoded toxin-antitoxin system HipAB.[17] A similar observation was previously made about the SXT-encoded toxin-antitoxin system MosAT.[22] While selective pressure and post-segregational killing (PSK) systems participate to the stability of R391, these functions are not conserved features of SXT/R391 ICEs and thus likely not conserved strategies for their stability.[7,17,22] Further experiments unraveled that replication and active-partition of the extrachromosomal ICE are important for its stability. The present commentary discusses the importance of these plasmid-like features in the global dynamics of ICEs.

Replication, A Key Step for ICEs Stability

The extrachromosomal form of ICEs was primarily assumed to be non-replicative besides the replication associated with conjugative transfer.[23] Since then, studies on various ICEs suspected that at least some ICEs might be capable of intracellular plasmid-like replication.[4,10,24-31] Recently, thorough studies on the biology of ICE*Bs1* of *Bacillus subtilis* demonstrated that this ICE conditionally replicates upon activation using a rolling-circle replication mechanism.[32,33] This replication uses the origin of transfer (*oriT*) of the element as the origin of replication and the relaxase as the replication initiator protein together with other ICE- and host-encoded components.[18] Biologically, this replication was shown to improve ICE*Bs1* stability, presumably by preventing its loss if the integration site is replicated while the element is excised (**Fig. 1**). Such a mechanism of ICE replication was proposed to be a common feature of many ICEs as they all carry an *oriT* and encode a relaxase.[17,34]

Seeking for the possible replication of R391 and SXT, we observed that they are present in multiple extrachromosomal copies in the subpopulation within which they are activated. Deletion of either the relaxase-encoding gene *traI* or the *oriT* of R391 strongly decreased the copy number of the element.[17] Decrease of the copy number of R391 strongly increased ICE loss, suggesting a key role of replication in the stability of SXT/R391 ICEs. SXT/R391 ICEs are integrated close to the origin of replication (*oriC*), both in the chromosome of *Escherichia coli* and in the chromosome 1 of *V. cholerae* (**Table 1**). In such fast-growing bacteria, rapid cell division is facilitated by the initiation of multiple replication forks during chromosome replication, i.e. between 6 and 14 replication forks per chromosome.[35,36] As a consequence, if the ICE excises prior to replication of its integration site, it will only be able to re-integrate into one of the 2 copies of its integration site, and thus will be lost in one of the 2 daughter cells after cell division (**Fig. 1**). Conditional replication likely prevents this issue by increasing the copy number of excised

Table 1. Chromosomal location of primary integration site(s) of a sample of ICEs

ICE name or family	Host	Integration sites[a]	Distance from the *oriC* (Mb)	Chromosome size (Mb)
SXT/R391	*Enterobacteriaceae* and *Vibrionaceae*	5′ end of the p*rfC* gene (*V. cholerae* chromosome 1)	0.7	2.7
		5′ end of the p*rfC* gene (*E. coli*)	0.7	4.6
ICE*Bs1*	*Bacillus subtilis*	3′ end of a tRNA(Leu) gene (*trnS-Leu2*)	0.5	4.2
ICE*St1*/ICE*St3*	*Streptococcus thermophilus*	3′ end of the *fda* gene	0.1	1.8
ICE_515_tRNA^{Lys}	*Streptococcus agalactiae*	3′ end of a tRNA(Lys) gene	0.1	2.1
RD2	*Streptococcus pyogenes*	3′ end of a tRNA(Thr) gene	0.6	1.9
ICE*clc*	*Pseudomonas putida*	3′ end of 4 tRNA(Gly) genes	1.6 / 2.1[b]	6.2
PAPI-1[c]/pKLC102	*Pseudomonas aeruginosa*	3′ end of a tRNA(Lys) gene	1.2	6.3
Tn5801	*Staphylococcus aureus*	3′ end of a gene encoding a GMP synthase	0.4	2.8
ICE*Lm1*	*Listeria monocytogenes*	3′ end of a gene encoding a GMP synthase	0.6	3.0
ICE*Mlo*$^{MAF-1}$	*Mesorhizobium loti*	3′ end of a tRNA(Phe) gene	1.8	7.0
ICE*Hin1056*	*Haemophilus influenzae*	3′ end of a tRNA(Leu) gene	0.1	1.9
CTnscr94	*Salmonella enterica*	3′ end of 2 tRNA(Phe) genes	1.7 / 0.3	4.8
SPI-7	*Salmonella enterica*	3′ end of a tRNA(Phe) gene	0.3	4.8
CW459Tet(M)	*Clostridium perfringens*	3′ End of a gene encoding a GMP synthase	0.4	3.0
Tn5397	*Clostridium diffcile*	CD630_04950	0.6	4.3

[a]Only the primary integration sites are indicated for each ICE.
[b]There are 3 consecutive integration sites at this locus.
[c]PAPI-1 can also integrate into the 3′ end of the other tRNA(Lys) located at 1.1 Mb away from *oriC* (PA0976.1).

ICE molecules concurrently with the increasing copy number of integration sites, thereby allowing reintegration in all the sites resulting from the replication of the initial single-copy one.

A rapid, yet non-exhaustive, analysis of the chromosomal integration sites of many known ICEs revealed that most integrate close to *oriC* in their host chromosome, including ICEs that were shown to replicate such as ICE*Bs1*, ICE*St3*, RD2 and ICE*Hin1056* (**Table 1**).[10,24,30,32] One could wonder why ICEs do integrate into chromosomal loci that tend to be close to *oriC*, and are thus more prone to be found in multiple copies per cell in fast-growing conditions. A plausible answer could be that numerous highly expressed essential genes are preferentially located close to *oriC*, at least in fast-growing bacteria.[35,37] Conserved genes are integration sites of choice for ICEs maintenance due to their high conservation between related bacterial species, thereby enhancing their host-range and success to establish and stably maintain in multiple bacterial lineages after conjugative transfer.[37,38]

Active Partition of ICEs

Further experiments aiming at unraveling other determinants of SXT/R391 ICEs stability revealed that all these ICEs code for SrpMRC (SXT/R391 partition), a functional type II (actin-like) active partition system.[17] As shown for the well-characterized system ParMRC of the plasmid R1, SrpMRC includes 3 components: a centromere-like region called *srpC*, a centromere-like binding protein called SrpR and an actin-like NTPase called SrpM.[17] Given the current knowledge on plasmid active partition system, SrpR is thought to bind to *srpC* and to recruit SrpM subunits that

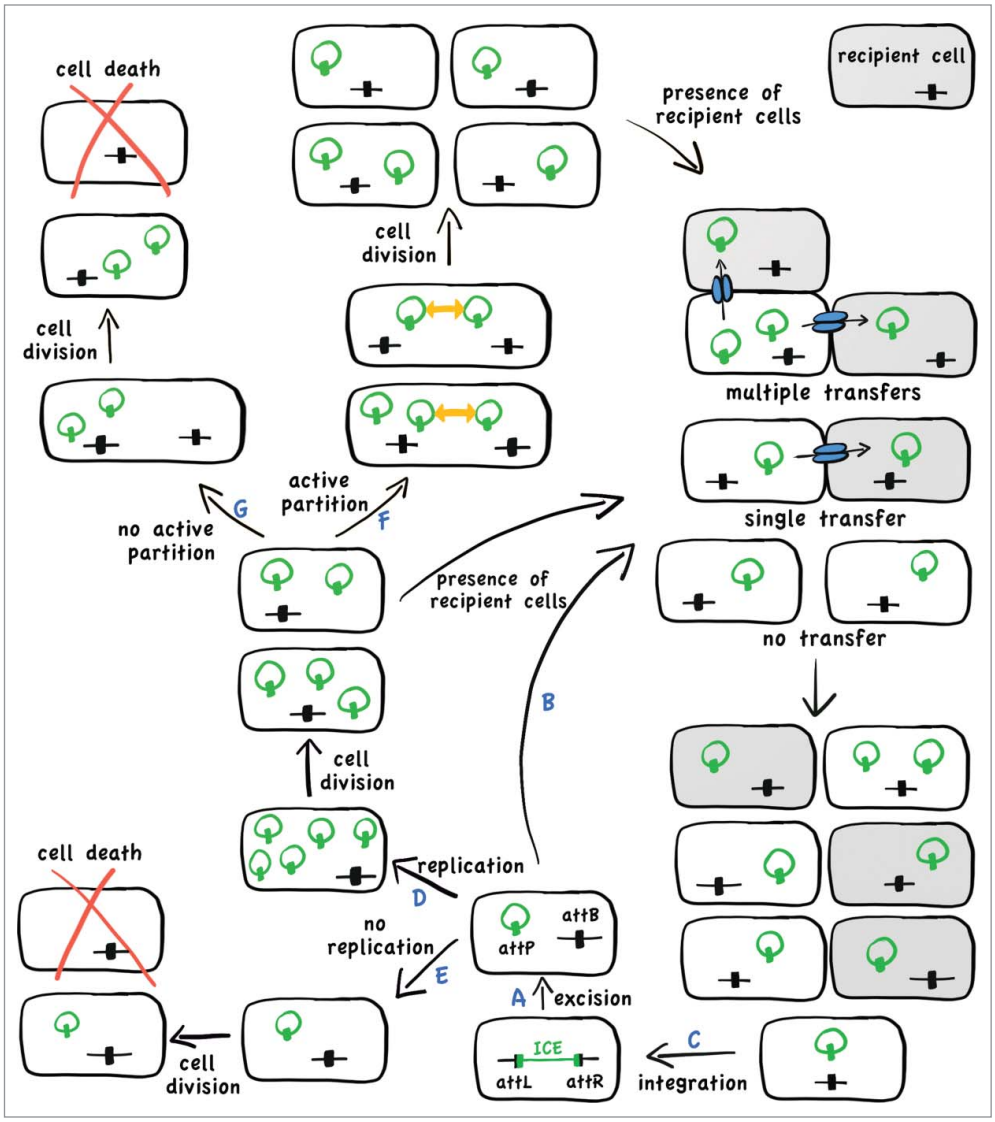

Figure 1. Schematic representation of the ICE lifecycle. At the bottom of the drawing, the quiescent ICE (green line) is integrated into a replicon of the host genome (black line). (**A**) Under conditions that activate the ICE, site-specific recombination between the *attL* and *attR* attachment sites that flank the ICE leads to its excision as an extrachromosomal circular molecule carrying an *attP* site (green circle with a green rectangle), and leaves an *attB* site (black line with a black rectangle). (**B**) In the presence of recipient cells (light gray filling), donor cells can undergo a single conjugative transfer event through an ICE-encoded T4SS (big blue ovals connecting 2 cells). (**C**) After conjugative transfer, the ICE integrates into the host genome by site-specific recombination between the *attP* and *attB* attachment sites. (**D**) Many ICEs are capable of intracellular plasmid-like replication to enhance their stability by allowing random repartition of the ICE copies during the cell division. (**E**) In the absence of replication, the extrachromosomal ICE could be lost if the integration site is replicated and the cell divides. Loss of the ICE likely promotes cell death due to ICE-encoded post-segregational killing systems (toxin-antitoxin or restriction-modification systems), or loss of adaptive traits (antibiotic or heavy metal resistances). In the presence of recipient cells, donor cells that contain one or multiple copies of the ICE could undergo single or multiples events of conjugative transfer. (**F**) Some ICEs may code for the machinery mediating the active partition of their replicated copies (orange double outward arrows linking 2 circular ICEs), ensuring equal repartition of ICE copies during the cell division. (**G**) If the copies are not distributed in the daughter cells, the ICE could be lost, leading to cell death. Donor cells containing one or multiple copies of the ICE could then undergo single or multiple events of conjugative transfer. Alternatively, ICEs could also replicate once in the recipient cell after conjugative transfer and/or begin another round of conjugative transfer before integrating into the host chromosome.

polymerize and push the ICE copies apart in the daughter cells during cell division (**Fig. 1**).[39] Interestingly, expression of *srpMR* depends on SetCD, the master activator of conjugative transfer functions, and thus is concomitant to *xis* and *int* expression, i.e., this system is expressed when the element excises and is vulnerable to loss.[17,19,40] As we reported in our *PLoS Genetics* article, other ICEs code for predicted active-partition systems, strongly suggesting that this feature has probably been overlooked in ICEs and could be more common than thought.[17,25,28,41-47]

An Updated Model of ICE Lifecycle

Given the recent experimental and *in silico* data collected from numerous studies on various ICEs, we propose an updated model of ICE lifecycle (**Fig. 1**). Accordingly to the original model, an ICE excises from its integration site and the resulting circular element transfers as a single-stranded DNA molecule toward a recipient cell by conjugation through the T4SS it encodes (**Fig. 1**). Once the transfer is completed, the ICE re-integrates in the chromosome of both the donor and recipient cells. Alternatively, many ICEs could be able to replicate when excised, thereby increasing their stability in case of replication of the integration site (**Fig. 1**). Each copy of the replicated element could then initiate an individual transfer event in the presence of recipient cells, likely increasing the frequency of transfer of the element. Some ICEs might also be able to actively segregate their copies using an active partition system, ensuring the correct repartition of the copies of the ICE during the cell division (**Fig. 1**).

In conclusion, while our understanding of ICEs biology becomes clearer, several questions remain to be elucidated. Is plasmid-like conditional replication really a universal feature of ICEs? How widespread is active partition among ICEs? Most studies on ICEs focus on adaptive traits conferred by these genomic islands rather than the mechanisms of their dissemination and maintenance among bacterial populations. A more systematic functional analysis of prototypical members of each ICEs family is needed to better understand the key features of their biology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Guglielmini J, Quintais L, Garcillán-Barcia MP, de la Cruz F, Rocha EPC. The repertoire of ICE in prokaryotes underscores the unity, diversity, and ubiquity of conjugation. PLoS Genet 2011; 7:e1002222; PMID:21876676; http://dx.doi.org/10.1371/journal.pgen.1002222
2. Carraro N, Sauvé M, Matteau D, Lauzon G, Rodrigue S, Burrus V. Development of pVCR94ΔX from Vibrio cholerae, a prototype for studying multidrug resistant IncA/C conjugative plasmids. Front Microbiol 2014; 5:44; PMID:24567731
3. Carraro N, Matteau D, Luo P, Rodrigue S, Burrus V. The master activator of IncA/C conjugative plasmids stimulates genomic islands and multidrug resistance dissemination. PLoS Genet 2014; 10:e1004714; PMID:25340549; http://dx.doi.org/10.1371/journal.pgen.1004714
4. Ramsay JP, Sullivan JT, Stuart GS, Lamont IL, Ronson CW. Excision and transfer of the Mesorhizobium loti R7A symbiosis island requires an integrase IntS, a novel recombination directionality factor RdfS, and a putative relaxase RlxS. Mol Microbiol 2006; 62:723-34; PMID:17076666; http://dx.doi.org/10.1111/j.1365-2958.2006.05396.x
5. Burrus V, Waldor MK. Shaping bacterial genomes with integrative and conjugative elements. Res Microbiol 2004; 155:376-86; PMID:15207870; http://dx.doi.org/10.1016/j.resmic.2004.01.012
6. Wozniak RAF, Waldor MK. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat Rev Microbiol 2010; 8:552-63; PMID:20601965; http://dx.doi.org/10.1038/nrmicro2382
7. Wozniak RAF, Fouts DE, Spagnoletti M, Colombo MM, Ceccarelli D, Garriss G, Déry C, Burrus V, Waldor MK. Comparative ICE genomics: insights into the evolution of the SXT/R391 family of ICEs. PLoS Genet 2009; 5:e1000786; PMID:20041216; http://dx.doi.org/10.1371/journal.pgen.1000786
8. Chuzeville S, Puymège A, Madec J-Y, Haenni M, Payot S. Characterization of a new CAMP factor carried by an integrative and conjugative element in Streptococcus agalactiae and spreading in Streptococci. PloS One 2012; 7:e48918; PMID:23152820; http://dx.doi.org/10.1371/journal.pone.0048918
9. Chuzeville S, Dramsi S, Madec J-Y, Haenni M, Payot S. Antigen I/II encoded by integrative and conjugative elements of Streptococcus agalactiae and role in biofilm formation. Microb Pathog 2015; 88:1-9; PMID:26232503; http://dx.doi.org/10.1016/j.micpath.2015.07.018
10. Sitkiewicz I, Green NM, Guo N, Mereghetti L, Musser JM. Lateral gene transfer of streptococcal ICE element RD2 (region of difference 2) encoding secreted proteins. BMC Microbiol 2011; 11:65; PMID:21457552; http://dx.doi.org/10.1186/1471-2180-11-65
11. He J, Baldini RL, Déziel E, Saucier M, Zhang Q, Liberati NT, Lee D, Urbach J, Goodman HM, Rahme LG. The broad host range pathogen Pseudomonas aeruginosa strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes. Proc Natl Acad Sci U S A 2004; 101:2530-5; PMID:14983043; http://dx.doi.org/10.1073/pnas.0304622101
12. Bellanger X, Payot S, Leblond-Bourget N, Guédon G. Conjugative and mobilizable genomic islands in bacteria: evolution and diversity. FEMS Microbiol Rev 2014; 38:720-60; PMID:24372381; http://dx.doi.org/10.1111/1574-6976.12058
13. Daccord A, Ceccarelli D, Burrus V. Integrating conjugative elements of the SXT/R391 family trigger the excision and drive the mobilization of a new class of Vibrio genomic islands. Mol Microbiol 2010; 78:576-88; PMID:20807202; http://dx.doi.org/10.1111/j.1365-2958.2010.07364.x
14. Daccord A, Ceccarelli D, Rodrigue S, Burrus V. Comparative analysis of mobilizable genomic islands. J Bacteriol 2013; 195:606-14; PMID:23204461; http://dx.doi.org/10.1128/JB.01985-12
15. Achard A, Leclercq R. Characterization of a small mobilizable transposon, MTnSag1, in Streptococcus agalactiae. J Bacteriol 2007; 189:4328-31; PMID:17416666; http://dx.doi.org/10.1128/JB.00213-07
16. Hochhut B, Marrero J, Waldor MK. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J Bacteriol 2000; 182:2043-7; PMID:10715015; http://dx.doi.org/10.1128/JB.182.7.2043-2047.2000
17. Carraro N, Poulin D, Burrus V. Replication and active partition of integrative and conjugative elements (ICEs) of the SXT/R391 family: the line between ICEs and conjugative plasmids is getting thinner. PLoS Genet 2015; 11:e1005298; PMID:26061412; http://dx.doi.org/10.1371/journal.pgen.1005298
18. Carraro N, Burrus V. Biology of Three ICE Families: SXT/R391, ICEBs1, and ICESt1/ICESt3. Microbiol Spectr 2014; 2:1-20; PMID:26104437
19. Poulin-Laprade D, Carraro N, Burrus V. The extended regulatory networks of SXT/R391 integrative and conjugative elements and IncA/C conjugative plasmids. Front Microbiol 2015; 6:837; PMID:26347724
20. Spagnoletti M, Ceccarelli D, Rieux A, Fondi M, Taviani E, Fani R, Colombo MM, Colwell RR, Balloux F. Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic Vibrio cholerae lineage. mBio 2014; 5:e01356-14; PMID:25139901; http://dx.doi.org/10.1128/mBio.01356-14
21. Burrus V, Marrero J, Waldor MK. The current ICE age: biology and evolution of SXT-related integrating conjugative elements. Plasmid 2006; 55:173-83; PMID:16530834; http://dx.doi.org/10.1016/j.plasmid.2006.01.001
22. Wozniak RAF, Waldor MK. A toxin-antitoxin system promotes the maintenance of an integrative conjugative element. PLoS Genet 2009; 5:e1000439; PMID:19325886; http://dx.doi.org/10.1371/journal.pgen.1000439
23. Burrus V, Pavlovic G, Decaris B, Guédon G. Conjugative transposons: the tip of the iceberg. Mol Microbiol 2002; 46:601-10; PMID:12410819; http://dx.doi.org/10.1046/j.1365-2958.2002.03191.x
24. Carraro N, Libante V, Morel C, Decaris B, Charron-Bourgoin F, Leblond P, Guedon G. Differential regulation of two closely related integrative and conjugative

elements from Streptococcus thermophilus. BMC Microbiol 2011; 11:238; PMID:22024428; http://dx.doi.org/10.1186/1471-2180-11-238
25. Guérillot R, Da Cunha V, Sauvage E, Bouchier C, Glaser P. Modular evolution of TnGBSs, a new family of integrative and conjugative elements associating insertion sequence transposition, plasmid replication, and conjugation for their spreading. J Bacteriol 2013; 195:1979-90; http://dx.doi.org/10.1128/JB.01745-12
26. Pembroke JT, Murphy DB. Isolation and analysis of a circular form of the IncJ conjugative transposon-like elements, R391 and R997: implications for IncJ incompatibility. FEMS Microbiol Lett 2000; 187:133-8; PMID:10856646; http://dx.doi.org/10.1111/j.1574-6968.2000.tb09149.x
27. Klockgether J, Würdemann D, Reva O, Wiehlmann L, Tümmler B. Diversity of the abundant pKLC102/PAGI-2 family of genomic islands in Pseudomonas aeruginosa. J Bacteriol 2007; 189:2443-59; PMID:17194795; http://dx.doi.org/10.1128/JB.01688-06
28. Klockgether J, Reva O, Larbig K, Tümmler B. Sequence analysis of the mobile genome island pKLC102 of Pseudomonas aeruginosa C. J Bacteriol 2004; 186:518-34; PMID:14702321; http://dx.doi.org/10.1128/JB.186.2.518-534.2004
29. Dimopoulou ID, Jordens JZ, Legakis NJ, Crook DW. A molecular analysis of Greek and UK Haemophilus influenzae conjugative resistance plasmids. J Antimicrob Chemother 1997; 39:303-7; PMID:9096178; http://dx.doi.org/10.1093/jac/39.3.303
30. Dimopoulou ID, Kraak WA, Anderson EC, Nichols WW, Slack MP, Crook DW. Molecular epidemiology of unrelated clusters of multiresistant strains of Haemophilus influenzae. J Infect Dis 1992; 165:1069-75; PMID:1583325; http://dx.doi.org/10.1093/infdis/165.6.1069
31. Leaves NI, Dimopoulou I, Hayes I, Kerridge S, Falla T, Secka O, Adegbola RA, Slack MP, Peto TE, Crook DW. Epidemiological studies of large resistance plasmids in Haemophilus. J Antimicrob Chemother 2000; 45:599-604; PMID:10797080; http://dx.doi.org/10.1093/jac/45.5.599
32. Lee CA, Babic A, Grossman AD. Autonomous plasmid-like replication of a conjugative transposon. Mol Microbiol 2010; 75:268-79; PMID:19943900; http://dx.doi.org/10.1111/j.1365-2958.2009.06985.x
33. Thomas J, Lee CA, Grossman AD. A conserved helicase processivity factor is needed for conjugation and replication of an integrative and conjugative element. PLoS Genet 2013; 9:e1003198; PMID:23326247; http://dx.doi.org/10.1371/journal.pgen.1003198
34. Grohmann E. Autonomous plasmid-like replication of Bacillus ICEBs1: a general feature of integrative conjugative elements? Mol Microbiol 2010; 75:261-3; PMID:19943906; http://dx.doi.org/10.1111/j.1365-2958.2009.06978.x
35. Couturier E, Rocha EPC. Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes. Mol Microbiol 2006; 59:1506-18; PMID:16468991; http://dx.doi.org/10.1111/j.1365-2958.2006.05046.x
36. Marians KJ. Mechanisms of replication fork restart in Escherichia coli. Philos Trans R Soc Lond B Biol Sci 2004; 359:71-7; PMID:15065658; http://dx.doi.org/10.1098/rstb.2003.1366
37. Rocha EPC. The replication-related organization of bacterial genomes. Microbiology 2004; 150:1609-27; PMID:15184548; http://dx.doi.org/10.1099/mic.0.26974-0
38. Touchon M, Bobay L-M, Rocha EP. The chromosomal accommodation and domestication of mobile genetic elements. Curr Opin Microbiol 2014; 22C:22-9; http://dx.doi.org/10.1016/j.mib.2014.09.010
39. Baxter JC, Funnell BE. Plasmid Partition Mechanisms. Microbiol Spectr 2014; 2:1-20; PMID:26104442; http://dx.doi.org/10.1128/microbiolspec.PLAS-0023-2014
40. Poulin-Laprade D, Matteau D, Jacques P-É, Rodrigue S, Burrus V. Transfer activation of SXT/R391 integrative and conjugative elements: unraveling the SetCD regulon. Nucleic Acids Res 2015; 43(4):2045-56: gkv071.
41. Dimopoulou ID, Russell JE, Mohd-Zain Z, Herbert R, Crook DW. Site-specific recombination with the chromosomal tRNA(Leu) gene by the large conjugative Haemophilus resistance plasmid. Antimicrob Agents Chemother 2002; 46:1602-3; PMID:11959612; http://dx.doi.org/10.1128/AAC.46.5.1602-1603.2002
42. Qiu X, Gurkar AU, Lory S. Interstrain transfer of the large pathogenicity island (PAPI-1) of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 2006; 103:19830-5; PMID:17179047; http://dx.doi.org/10.1073/pnas.0606810104
43. Juhas M, Power PM, Harding RM, Ferguson DJP, Dimopoulou ID, Elamin AR e, Mohd-Zain Z, Hood DW, Adegbola R, Erwin A, et al. Sequence and functional analyses of Haemophilus spp. genomic islands. Genome Biol 2007; 8:R237; PMID:17996041; http://dx.doi.org/10.1186/gb-2007-8-11-r237
44. Mohd-Zain Z, Turner SL, Cerdeño-Tárraga AM, Lilley AK, Inzana TJ, Duncan AJ, Harding RM, Hood DW, Peto TE, Crook DW. Transferable antibiotic resistance elements in Haemophilus influenzae share a common evolutionary origin with a diverse family of syntenic genomic islands. J Bacteriol 2004; 186:8114-22; PMID:15547285; http://dx.doi.org/10.1128/JB.186.23.8114-8122.2004
45. Ryan MP, Pembroke JT, Adley CC. Novel Tn4371-ICE like element in Ralstonia pickettii and genome mining for comparative elements. BMC Microbiol 2009; 9:242; PMID:19941653; http://dx.doi.org/10.1186/1471-2180-9-242
46. She Q, Phan H, Garrett RA, Albers SV, Stedman KM, Zillig W. Genetic profile of pNOB8 from Sulfolobus: the first conjugative plasmid from an archaeon. Extrem Life Extreme Cond 1998; 2:417-25; http://dx.doi.org/10.1007/s007920050087
47. Marenda M, Barbe V, Gourgues G, Mangenot S, Sagne E, Citti C. A new integrative conjugative element occurs in Mycoplasma agalactiae as chromosomal and free circular forms. J Bacteriol 2006; 188:4137-41; PMID:16707706; http://dx.doi.org/10.1128/JB.00114-06