Mobilizable genomic islands, different strategies for the dissemination of multidrug resistance and other adaptive traits

Nicolas Carraro, Nicolas Rivard, Vincent Burrus, and Daniela Ceccarelli

Laboratory of Bacterial Molecular Genetics, Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada; Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; Department of Bacteriology and Epidemiology, Wageningen Bioveterinary Research, Lelystad, the Netherlands

ABSTRACT

Mobile genetic elements are near ubiquitous DNA segments that revealed a surprising variety of strategies for their propagation among prokaryotes and between eukaryotes. In bacteria, conjugative elements were shown to be key drivers of evolution and adaptation by efficiently disseminating genes involved in pathogenicity, symbiosis, metabolic pathways, and antibiotic resistance. Conjugative plasmids of the incompatibility groups A and C (A/C) are important vehicles for the dissemination of antibiotic resistance and the consequent global emergence and spread of multi-resistant pathogenic bacteria. Beyond their own mobility, A/C plasmids were also shown to drive the mobility of unrelated non-autonomous mobilizable genomic islands, which may also confer further advantageous traits. In this commentary, we summarize the current knowledge on different classes of A/C-dependent mobilizable genomic islands and we discuss other DNA hitchhikers and their implication in bacterial evolution. Furthermore, we glimpse at the complex genetic network linking autonomous and non-autonomous mobile genetic elements, and at the associated flow of genetic information between bacteria.

KEYWORDS

A/C; antibiotic resistance; genomic island; integrative conjugative element; mobilization; plasmid

Bacterial genomes are dynamic entities subjected to a constant flow of loss and gain of genetic material. Gene acquisition can provide bacterial hosts with adaptive traits and is likely to confer a selective advantage in particular conditions. Self-transmissible mobile genetic elements (MGEs) such as prophages and conjugative elements were shown to be an immense resource for genome evolution and bacterial adaptation.

Genomic islands (GIs) also largely participate in bacterial genome diversification. Many GIs were identified thanks to the adaptive traits they encoded and named accordingly, e.g. pathogenicity islands or symbiosis islands. GIs are chromosomal DNA segments typically present in subsets of closely related strains, one of the hallmarks of acquisition by horizontal gene transfer. However, the mechanism of acquisition and dissemination of GIs has remained a conundrum for a long time, often due to the lack of obvious mobility–related genes. Recent studies have refined our understanding of the biology and mobility mechanisms of several of mobilizable GIs (MGIs) families including satellite prophages and GIs mobilized by conjugative elements. Mobility of MGIs involves self-transmissible MGEs that provide them with functions they lack to catalyze their dissemination. Availability of thousands of bacterial genome sequences associated with low-cost, high-throughput modern molecular methods unraveled an even greater diversity of GIs.

We recently reported the discovery of MGI_{Vch-Hai6}, a new mobile resistance island in *Vibrio cholerae*, that is mobilizable by A/C conjugative plasmids. Here, we compare the possible mechanisms of activation and mobilization of MGI_{Vch-Hai6} with two other A/C-dependent mobilizable GIs as well as a family of GIs mobilized by integrative and conjugative elements (ICEs) of the SXT/R391 family.
Regulation of transfer of A/C plasmids

Plasmids of the incompatibility groups A and C (A/C) are large (> 110 kb) double-stranded molecules that efficiently disseminate by conjugation. A/C plasmids drive the spread of multiple antibiotic resistances including last-resort antimicrobial compounds such as carbapenems. 

Recent studies demonstrated that control of A/C plasmids mobility is reminiscent of the FlhCD-dependent activation of flagellar motility in *Escherichia coli* and related motile bacteria. Nevertheless, the A/C regulatory circuitry is a unique system with specific early molecular actors and plasmid-borne target genes. Two repressors named Acr1 and Acr2 (A/C repressors 1 and 2) repress the constitutive transcription of *acr1* from *Pacr1*. Upon inducing conditions that remain to be identified, repression of *Pacr1* is alleviated, allowing not only the transcription of *acr1* but also that of all four downstream genes. Two of these genes, *acaC* and *acaD*, were shown to code for subunits of the FlhCD-like master activator of A/C plasmids AcaCD (A/C activator, subunits C and D). Thorough investigation revealed that AcaCD targets 18 A/C-borne promoter regions, thereby activating the transcription of genes and operons responsible for conjugative transfer. Surprisingly, genes coding for predicted or demonstrated functions account for a fraction of AcaCD-activated genes as nearly two thirds of these genes code for proteins of unknown function. Future investigation of this large *terra incognita* is necessary to fully understand the biology of A/C plasmids.

A/C-dependent mobilization of mobilizable genomic islands

Recent work conducted by our group uncovered the extended role of AcaCD. Besides A/C-borne sequences, AcaCD was shown to recognize chromosomal loci that belong to A/C-unrelated MGIs.

To date, three unrelated families of A/C-dependent MGIs were identified and named after the prototypical elements that were experimentally characterized: MGI*VchHai6* of *V. cholerae*, MGI*Vmi1* of *V. mimicus* and *Salmonella* genomic island 1 (SGI1). Each family encompasses several members sharing a conserved core sequence. Different members of the same family contain distinct insertion of variable DNA coding for adaptive traits or proteins of unknown function. While the precise molecular mechanism leading to intercellular mobility of these elements remains to be deciphered, accumulation of evidence suggests the pivotal role of genes under the control of AcaCD.

Based on these observations and the presence of conserved features, we propose two distinct models of mobilization, one for SGI1 and relatives, and the other for MGIVchHai6/MGIVmi1-like elements (Fig. 1).

**SGI1: First evidence of A/C-dependent MGI**

A/C were firstly shown to specifically mobilize GIs in 2005 with the characterization of SGI1 and by extent...
the large family of SGI1 elements.\textsuperscript{13,16,20} SGI1 and its siblings are recognized as major determinants of multidrug resistance in \textit{Salmonella enterica} and \textit{Proteus mirabilis}.\textsuperscript{18} The integron In104 of SGI1 elements confers multidrug resistance.\textsuperscript{18,19}

Five AcaCD binding sites were discovered in SGI1, allowing to posit on the underlying mechanism allowing SGI1 mobilization (Fig. 1A).\textsuperscript{13,21,22} SGI1 carries \textit{int}, a constitutively expressed gene coding for the site-specific integrase that catalyzes the integration of SGI1 into the 3' end of \textit{trmE} in the chromosome of its host.\textsuperscript{16,21,23,24} Upon arrival of an A/C plasmid in the cell, associated synthesis of AcaCD triggers the transcription of the RDF gene \textit{xis} and of a gene coding for a Mobi-like protein (Fig. 1B). Mobi is required for transfer of integrative and conjugative elements (ICEs) of the SXT/R391 family and A/C plasmids.\textsuperscript{26,27} In SXT/R391 ICEs and A/C plasmids, \textit{oriT} is located in a large intergenic region upstream of \textit{mobi}.\textsuperscript{26,27} By analogy, we predict that \textit{oriT} of MGI\textit{VchHai6/MGIVmi1} elements (\textit{oriT}_{\text{MGI}}) is located in the large intergenic region upstream of \textit{mobi}_{\text{MGI}} (Fig. 1B).

A model for MGI\textit{VchHai6/MGIVmi1} lifecycle infers that these elements remain quiescent in their integrated chromosomal state. Based on work done on other MGIs, stable integration of MGI\textit{VchHai6/MGIVmi1} elements is likely enabled by constitutive expression of \textit{int}.\textsuperscript{21,28} Like for SGI1, entry of an A/C plasmid that expresses AcaCD triggers the synthesis of Xis that, in concert with the integrase, mediates the excision of the MGI (Fig. 1B). AcaCD also activates the synthesis of MobI\textsubscript{MGI} that is thought to recognize and bind to \textit{oriT}_{\text{MGI}}.\textsuperscript{26,27} MobI\textsubscript{MGI} would act as an adaptor protein that recruits and assembles the A/C plasmid encoded DNA-processing machinery, called relaxosome, within which the relaxase TraI initiates conjugative transfer through the A/C-encoded mating pore (Fig. 1B). The MGI is then assumed to be able to site-specifically integrate into the genome of the recipient cell regardless of the presence of the A/C plasmid, as expression of the integrase is constitutive.

**Members of the MGI\textit{VchHai6} family are mobilizable by A/C plasmids**

MGI\textit{VchHai6} is the prototypical member of a new family of MGIs involved in the dissemination of multidrug resistance.\textsuperscript{8} This 47-kb element was identified in a non-O1/non-O139 \textit{V. cholerae} clinical isolate recovered from a cholera patient in Haiti in 2010.\textsuperscript{25} Like SGI1, MGI\textit{VchHai6} is integrated into the 3' end of \textit{trmE}. It also carries a distinct integron, In36A1, conferring resistance to \textit{β}-lactams, florfenicol/chloramphenicol, streptomycin/spectinomycin, sulfamethoxazole and trimethoprim (co-trimoxazole), and tetracycline. MGI\textit{VchHai6} also likely confers resistance to bacteriophage infection and mercury, as it bears a type 1 restriction-modification system and Tn6310. MGI\textit{VchHai6}-like elements are globally distributed in environmental and clinical \textit{V. cholerae} isolates recovered from 1977 to 2010. All members of this family of MGIs share a ~8-kb conserved core that likely ensures essential maintenance and transfer functions. Mobility of MGI\textit{VchHai6} was shown to be strictly dependent on the presence of an A/C plasmid.

Despite its different size and gene content, MGI\textit{VchHai6} shares several features with MGI\textit{Vmi1}, an element integrated into the 3' end of \textit{yicC}.\textsuperscript{8,13,15} In both MGI\textit{VchHai6} and MGI\textit{Vmi1}, AcaCD was shown to drive the transcription of the RDF gene \textit{xis} and of a gene coding for a Mobi-like protein (Fig. 1B). Mobi is required for transfer of integrative and conjugative elements (ICEs) of the SXT/R391 family and A/C plasmids.\textsuperscript{26,27} In SXT/R391 ICEs and A/C plasmids, \textit{oriT} is located in a large intergenic region upstream of \textit{mobi}.\textsuperscript{26,27}

**MGIs come in many flavors**

For several decades, studies of members of well-known families of self-transmissible MGEs eclipsed the discovery of new types of mobile elements. New evidence suggests that MGIs could be more abundant in regards to their site-specific integration due to the constitutive expression of \textit{int}.\textsuperscript{21}
Early milestones into the discovery of MGIs include the characterization of CTn-dependent non-replicating Bacteroides units (NBUs), phage-mobilizable Staphylococcus aureus pathogenicity islands (SaPIs), and the mobilizable transposon of Streptococcus agalactiae MTnSag1, whose mobility depends on Tn916.\(^5,30-36\) The identification of oriT\(_\text{SXT}\) and elucidation of the master activator SetCD of SXT/R391 ICEs also greatly helped our recent investigation on A/C-dependent MGIs.\(^{26,37}\) Indeed, localization of oriT\(_\text{SXT}\) allowed the identification of similar chromosomal loci.\(^{38}\) Further investigations revealed that these chromosomal oriT sequences belonged to integrated MGIs, whose mobilization mechanism is slightly different from the above-described MGIs (Fig. 1C).\(^{12,28,37-39}\) Excision of these elements depends on the SetCD-dependent transcriptional activation of the RDF gene.\(^{12,28,37}\) oriT\(_\text{MGII}\) mimics oriT\(_\text{SXT}\) hence it is recognized by Mob\(_\text{SXT}\) and processed by the ICE-encoded relaxosome prior to transfer through the ICE-encoded mating pore (Fig. 1C). In the recipient cell, the MGI integrates autonomously due to int constitutive expression.\(^{12,28}\)

**Concluding remarks**

The propensity of MGIs to persist into and disseminate between bacterial populations using diverse strategies indicates that they are not defective elements. MGIs have rather adapted to act as parasites of self-transmissible MGEs, while at the same time spreading adaptive traits such as resistance to multiple antimicrobial compounds. An interesting example of this cooperative/antagonistic relationship is provided by SGI1 and variants that rely on IncC plasmids for mobilization.\(^{16}\) Co-transfer of plasmid and GI is rare suggesting that the latter is able to affect plasmid transfer, as also confirmed by the rapid loss of the plasmid when SGI1 is co-present in the same E. coli cell.\(^{40}\)

This captivating research area is likely to deepen our understanding of other families of MGEs, including other classes of MGIs. Future investigations must focus on (i) the characterization of master regulators and cognate target sequences of a broad set of self-transmissible MGEs and (ii) the identification of their oriT sequence. Such valuable information will help when performing data mining of genome sequences to identify new DNA elements acquired by horizontal gene transfer. In-depth, step-by-step discoveries will help paving the road to building an atlas of interconnections between MGEs and associated massive flow of genetic material.

**Abbreviations**

A/C plasmids of incompatibility groups A and C  
GI genomic island  
ICE integrative conjugative element  
MGE mobile genetic element  
MGI mobilizable genomic island  
oriT origin of transfer

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Funding**

This work was supported by a Discovery Grant [2016-04365] from the Natural Sciences and Engineering Council of Canada (NSERC) to VB.

**ORCID**

Daniela Ceccarelli http://orcid.org/0000-0001-7502-5438

**References**

[1] Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: building the web of life. Nat Rev Genet 2015; 16:472-82; PMID:26184597; http://dx.doi.org/10.1038/nrg3962

[2] Koonin EV. Horizontal gene transfer: essentiality and evolvability in prokaryotes, and roles in evolutionary transitions. F1000Research 2016; 5; PMID:27508073; http://dx.doi.org/10.12688/f1000research.8737.1

[3] Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 2005; 3:722-32; PMID:16138100; http://dx.doi.org/10.1038/nrmicro1235

[4] Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW. Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol Rev 2009; 33:376-93; PMID:19178566; http://dx.doi.org/10.1111/j.1574-6976.2008.00136.x

[5] 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

[6] Lee CA, Thomas J, Grossman AD. The Bacillus subtilis conjugative transposon ICEBs1 mobilizes plasmids lacking dedicated mobilization functions. J Bacteriol 2012;
Wailan AM, Sartor AL, Zowawi HM, Perry JD, Paterson DL, Sidjabat HE. Genetic contexts of blaNDM-1 in patients carrying multiple NDM-producing strains. Antimicrob Agents Chemother 2015; 59:7405-10; PMID:26392493; http://dx.doi.org/10.1128/AAC.01319-15

Harmer CJ, Hall RM. The A to Z of A/C plasmids. Plasmid 2015; 80:63-82; PMID:25910948; http://dx.doi.org/10.1016/j.plasmid.2015.04.003

Wailan AM, Sartor AL, Zowawi HM, Perry JD, Paterson DL, Sidjabat HE. Genetic contexts of blaNDM-1 in patients carrying multiple NDM-producing strains. Antimicrob Agents Chemother 2015; 59:7405-10; PMID:26392493; http://dx.doi.org/10.1128/AAC.01319-15

Harmer CJ, Hall RM. pRMH760, a precursor of A/C plasmids. Plasmid 2016; 87-88:17-27; PMID:27492737; http://dx.doi.org/10.1016/j.plasmid.2015.04.003

Carraro N, Rivard N, Ceccarelli D, Colwell RR, Burrus V. The extended regulatory network of IncA/C conjugative plasmids mobilizes a new family of multidrug resistance islands in clinical Vibrio cholerae non-O1/non-O139 isolates from Haiti. mBio 2016; 7(4): e00509-16; PMID:27435459; http://dx.doi.org/10.1128/mBio.00509-16

Harmer CJ, Hall RM. The A to Z of A/C plasmids. Plasmid 2015; 80:63-82; PMID:25910948; http://dx.doi.org/10.1016/j.plasmid.2015.04.003

Carraro N, Sauvé M, Matteau D, Lauzon G, Rodrigue S, Burrus V. Development of pVC9R4AX from Vibrio cholerae, a prototype for studying multidrug resistant IncA/C conjugative plasmids. Front Microbiol 2014; 5:44; PMID:24567731; http://dx.doi.org/10.3389/fmicb.2015.00837

Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP. The genomic island SG1, containing the multiple antibiotic resistance region of Salmonella enterica serovar Typhimurium DT104 or variants of it, is widely distributed in other S. enterica serovars. J Bacteriol 2005; 187:4401-9; PMID:15968049; http://dx.doi.org/10.1128/JB.187.13.4401-4409.2005

Hall RM. Salmonella genomic islands and antibiotic resistance in Salmonella enterica. Future Microbiol 2010; 5:1525-38; PMID:21073312; http://dx.doi.org/10.2217/fmb.10.122

Boyd DA, Shi X, Hu Q, Ng LK, Doublet B, Cloeckaert A, Mulvey MR. Salmonella genomic island 1 (SG1), variant SG1-I, and new variant SG1-I-O in Proteus mirabilis clinical and food isolates from China. Antimicrob Agents Chemother 2008; 52:340-4; PMID:18025121; http://dx.doi.org/10.1128/AAC.00902-07

Douard G, Praud K, Cloeckaert A, Doublet B. The Salmonella genomic island 1 is specifically mobilized in trans by the IncA/C multidrug resistance plasmid family. PloS One 2010; 5:e15302; PMID:21187963; http://dx.doi.org/10.1371/journal.pone.0015302

Kiss J, Papp PP, Szabó M, Farkas T, Murányi G, Szakállas E, Olasz F. The master regulator of IncA/C plasmids is recognized by the Salmonella genomic island SG1 as a signal for excision and conjugal transfer. Nucleic Acids Res 2015; 43:8735-45; PMID:26209134; http://dx.doi.org/10.1093/nar/gkv758

Murányi G, Szabó M, Olasz F, Kiss J. Determination and analysis of the putative AcaCD-responsive promoters of Salmonella genomic island 1. PloS One 2016; 11: e0164561; PMID:27727307; http://dx.doi.org/10.1371/journal.pone.0164561

Mulvey MR, Boyd DA, Olson AB, Doublet B, Cloeckaert A. The genetics of Salmonella genomic island 1. Microbes Infect Inst Pasteur 2006; 8:1915-22; http://dx.doi.org/10.1016/j.micinf.2005.12.028

Kiss J, Nagy B, Olasz F. Stability, entrapment and variant formation of Salmonella genomic island 1. PloS One 2012; 7:e32497; PMID:22384263; http://dx.doi.org/10.1371/journal.pone.0032497

Hasan NA, Choi SY, Eppinger M, Clark PW, Chen A, Alam M, Haley BJ, Taviani E, Hine E, Su Q, et al. Genomic diversity of 2010 Haitian cholera outbreak strains. Proc Natl Acad Sci U S A 2012; 109:E2010-2017; PMID:22711841; http://dx.doi.org/10.1073/pnas.1207359109

Kiss J, Nagy B, Olasz F. Stability, entrapment and variant formation of Salmonella genomic island 1. PloS One 2012; 7:e32497; PMID:22384263; http://dx.doi.org/10.1371/journal.pone.0032497

Carraro N, Sauvé M, Matteau D, Lauzon G, Rodrigue S, Burrus V. Development of pVC9R4AX from Vibrio cholerae, a prototype for studying multidrug resistant IncA/C conjugative plasmids. Front Microbiol 2014; 5:44; PMID:24567731; http://dx.doi.org/10.3389/fmicb.2015.00837
[30] Shoemaker NB, Wang GR, Salyers AA. NBU1, a mobilizable site-specific integrated element from Bacteroides spp., can integrate nonspecifically in Escherichia coli. J Bacteriol 1996; 178:3601-7; PMID:8655560; http://dx.doi.org/10.1128/jb.178.12.3601-3607.1996

[31] Kreiswirth BN, Projan SJ, Schlievert PM, Novick RP. Toxic shock syndrome toxin 1 is encoded by a variable genetic element. Rev Infect Dis 1989; 11(Suppl 1):S83-88; discussion S88-89; PMID:2564693; http://dx.doi.org/10.1093/clinids/11.Supplement_1.S83

[32] Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in Staphylococcus aureus. Mol Microbiol 1998; 29:527-43; PMID:9720870; http://dx.doi.org/10.1046/j.1365-2958.1998.00947.x

[33] Ruzin A, Lindsay J, Novick RP. Molecular genetics of SaPI1—a mobile pathogenicity island in Staphylococcus aureus. Mol Microbiol 2001; 41:365-77; PMID:11489124; http://dx.doi.org/10.1046/j.1365-2958.2001.02488.x

[34] Novick RP, Christie GE, Penadés JR. The phage-related chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol 2010; 8:541-51; PMID:20634809; http://dx.doi.org/10.1038/nrmicro2393

[35] Martínez-Rubio R, Quiles-Puchalt N, Martí M, Humphrey S, Ram G, Smyth D, Chen J, Novick RP, Penadés JR. Phage-inducible islands in the Gram-positive cocci. ISME J 2016; PMID:27959343; http://dx.doi.org/10.1038/ismej.2016.163

[36] 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

[37] 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; PMID:25662215; http://dx.doi.org/10.1093/nar/gkv071

[38] 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

[39] 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

[40] Harmer CJ, Hamidian M, Ambrose SJ, Hall RM. Destabilization of IncA and IncC plasmids by SGI1 and SGI2 type Salmonella genomic islands. Plasmid 2016; 87-88:51-57; PMID:27620651; http://dx.doi.org/10.1016/j.plasmid.2016.09.003