Mobile Genetic Elements 3:2, e24774; March/April 2013; © 2013 Landes Bioscience

# Costly Class-1 integrons and the domestication of the the functional integrase

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**Keywords:** Class-1 integrons, fitness cost of resistance

Class-1 integrons play an important role in the emergence and spread of antimicrobial resistance determinants. In a recent study we showed that host fitness was dramatically reduced following acquisition of these elements. These fitness costs were due to the presence of an active integrase and we suggested that the mechanistic explanation was due to reduced genetic stability through IntI1 mediated recombination events between *attI*/*attC* and non-canonical sites in the chromosome. Here we demonstrate that the *attI* degenerated target sequence is highly prevalent in our model organism *Acinetobacter baylyi* adding support to the hypothesis that IntI1 is costly due to genomic instability.

Mobile genetic elements (MGEs) are key players in the emergence and spread of adaptive traits in bacterial populations. When they move horizontally they can provide ready-to-use genes, operons, and even clusters of genes such as integrons, conjugative transposons, and genetic islands already pre-adapted in other strains and species.[1] When compared with genome evolution limited to processes within the single cell, primarily driven by the slow accumulation of rare point mutations, deletions, and gene-amplification events,[2] it is clear that successful acquisition of MGEs can provide giant leaps in bacterial adaptation to for example environmental stressors. Nowhere is this more evident than the rapid emergence and spread of antibiotic resistance determinants residing on various MGEs in clinically relevant bacterial species. Yet, with the exception of plasmids,[3-6] surprisingly few studies have addressed the impact MGE acquisitions on the population dynamics of the bacterial host or the subsequent co-evolutionary trajectories following successful transfer events. Such research is important from the perspective of both emergence and reversal of antibiotic resistance determinants.[7,8] One class of MGE's where data on the effects on the population dynamics of the novel host is limited is the ubiquitous integrons.

Integrons were discovered in the late 1980s[9] and these genetic elements encode an integrase (IntI) that enables site-specific integration and excision of gene cassettes, among the smallest MGEs currently described.[10] The integrase gene is located in the 5'-conserved region of the integron and enables site-specific recombination between the gene-cassette-borne *attC* site (also known as the "59-base pair element") and the primary recombination site *attI* present immediately adjacent to *intI*. Captured gene cassettes are typically transcribed from the gene cassette promoter ($P_C$) embedded within the *intI*.[11,12] Expression of *intI* is controlled by SOS boxes (LexA binding sites) overlapping with the integrase promoter $P_{intI}$.[13,14] The structure of the 3'-conserved region usually includes a truncated *qacE1* (resistance to quaternary ammonium compounds), a *sul1* gene (sulphonamide-resistance), and one or two open reading frames (ORFs) encoding hypothetical proteins of unknown functions.[11] Integrons involved in antibiotic resistance dissemination, the so-called mobile integrons, are divided into five main classes (Class1–5) based on the *intI* sequence.[10]

In a recent report[15] we asked if acquisition of Class-1 integrons (the most widespread and best described integron-class) would affect bacterial fitness. We inserted three Class-1 integrons from two *Acinetobacter baumannii* and one *Salmonella enterica* Serovar Typhimurium clinical isolates into a predefined selectively neutral chromosomal site in *Acinetobacter baylyi*. All transferred integrons severely reduced host fitness from 7 to 11%. Importantly, *A. baylyi* and the more clinically relevant *A. baumannii* lack *lexA* homologs[16,17] and in these strains the *intI1* gene is most likely not regulated by the SOS response as recently described,[13,14] presumably allowing for constitutive expression of this gene. Such fitness costs are comparable to chromosomal mutations in housekeeping genes conferring resistances to various antimicrobial agents.[18,19] We hypothesized that either the active IntI1 and/or expression of gene cassettes were responsible for the observed fitness effects following acquisition of the three integrons. A closer inspection of our data suggested that the gene-cassette content was most likely not responsible for the reduction of bacterial fitness since no apparent correlation was seen between the number of gene-cassettes and impact on fitness. We then insertionally inactivated the integrases in all three integron containing *A. baylyi* strains and confirmed loss of *intI1* expression by

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Submitted: 03/29/13; Revised: 04/15/13; Accepted: 04/22/13
Citation: Harms K, Starikova I, Johnsen PJ. Costly Class-1 integrons and the domestication of the the functional integrase. Mobile Genetic Elements 2013; 3:e24774; http://dx.doi.org/10.4161/mge.24774

**Table 1.** Presence of *attI* core sequences in selected bacterial chromosomes

| Species | Examined chromosomes | Ratio of observed to expected number of attI sites per chromosome | |
|---|---|---|---|
| | | Median | Range |
| *Acinetobacter baylyi* | 1[a] | 1.002 | - |
| *Acinetobacter baumannii* | 12[b] | 1.223 | 1.212–1.227 |
| *Pseudomonas aeruginosa* | 11[c] | 0.387 | 0.373–0.401 |
| *Salmonella enterica* serovar Typhimurium | 8[d] | 0.791 | 0.785–0.793 |
| *Klebsiella pneumoniae* | 7[e] | 0.607 | 0.600–0.611 |
| *Escherichia coli* | 10[f] | 0.825 | 0.794–0.833 |

All complete sequences (as of April 4th 2013) were analyzed for: *A. baumannii*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp enterica serovar Typhimurium, *Klebsiella pneumoniae* as well as 10 randomly chosen *E. coli genomes*. GenBank accession numbers: [a]NC005966; [b]NC009085, NC010611, NC010400, NC010410, NC011568, NC011595, NC017162, NC017171, NC017387, NC017847, NC018706, and NC020547; [c]AE004901, AFXJ01000001, AFXK01000001, NC008463, NC009656, NC011770, NC017548, NC017549, NC018080, NZ_AAQW01000001, and NZ_CM001020; [d]NC003179, NC016810, NC016854, NC016856, NC016857, NC016860, NC016863, and NC017046; [e]NC009648, NC011283, NC012731, NC016845, NC017540, NC018522, and NZ_APGM01000001; [f]NC000913, NC002695, NC010498, NC011601, NC011742, NC012967, NC013008, NC017633, NC017635, and NC017651.

reverse transcription PCR. Subsequent measurements of relative fitness between these knockout mutants and the integron-free *A. baylyi* revealed that the initial fitness costs of harbouring an integron were completely mitigated in all three mutants. These results provide clear evidence that the fitness cost of integron carriage was due to the presence of an active integrase. Subsequent analyses of the $P_C$ regions (embedded in the *intI1*) revealed a weak correlation between the reported strengths of the gene-cassette promoters[20] and differences in relative fitness between otherwise isogenic integron-containing and -free *A. baylyi*. The integron containing the strongest $P_C$ reduced fitness significantly more than the integron containing the weakest $P_C$. Taken together with recent reports showing an inverse correlation between $P_C$ strength and both *intI1*-activity[20,21] and -expression levels[22] our results favor the hypothesis that integrase expression levels directly affect host fitness.

Two of the three integrons transferred from clinical isolates into *A. baylyi* contained LexA binding sites in the *intI1* promoter regions. As pointed out above, no *lexA* homologs are present in several members of the *Acinetobacter* genus, including *A. baylyi*. The presented data in[15] clearly demonstrates the need for tight regulation of integron integrases. Moreover, the lack of biological costs of integrons containing inactivated *intI1* genes could very well mimic the effects on host fitness in species where LexA represses the SOS response as well as the transcription of integrases. However, as demonstrated in[14] the integrase is still transcribed at low levels when repressed by LexA and a small effect of host fitness may be expected. Thus, we argue that the described fitness costs of *intI1* can partly explain the high frequencies of pseudo-integrases in bacterial population surveys as seen in.[13,23,24] We also demonstrated that inactivated integrases emerged during continuous culture experiments with mutational inactivation patterns similar to those described in the literature, providing further evidence in support of this hypothesis.[15]

We proposed that the most likely mechanistic basis for the "costly" *intI1* is reduced genomic stability. This was due to previous experimental reports demonstrating that IntI1 can mediate recombination events between *attI*/*attC* and non-canonical sites in host DNA.[25] Moreover, two recent reports demonstrated that purified IntI1 possesses all necessary functions for target site recognition and recombination.[26,27] It is thus clear that IntI1 can form recombination junctions between the Class-1 integron and sites elsewhere in the genome, and that resolutions of these structures can lead to potentially abortive recombination events like deleterious deletions of whole chromosomal regions. Early searches for target sequences recognized by IntI1 within the gene cassettes revealed little sequence conservation, but a specific short degenerated sequence, the core site (consensus GTTRRRY), appears to always be present and recombination occurs between the G and the first T residue.[28] To further support our mechanistic explanation we counted the number of the GTTRRRY sequence motif in the genome of *A. baylyi* ADP1 (3,598,621 base pairs) using the pDraw program (http://acaclone.com) and compared it with the expected number of this motif in randomly composed DNA calculated as follows: The genome size was divided by the frequency of GTTRRRY ($4^3 \times 2^4$) and multiplied by 2 for the reverse complement. Overall, the *attI* core consensus motif is expected to occur once every 512 base pairs. For *A. baylyi* ADP1, the observed frequency (7,040 sites counted) was as expected (7,029) (**Table 1**). We extended the analyses to additional species with full chromosomal sequences deposited in GenBank, including *A. baumannii*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp enterica serovar Typhimurium, *Klebsiella pneumoniae* (all complete sequences available as of April 4th 2013) and *Escherichia coli* (ten randomly taken sequences). The results are presented in **Table 1** and indicate that the prevalence of *attI* sites is as expected only in *A. baumannii* but somewhat lower in the other species examined. On the other hand, even when the observed frequency of *attI* core sites is only about 0.387 (in *P. aeruginosa*), there are more than 4,500 *attI* core sites present in every chromosome. The presented findings further underscore the need for tight regulation of IntI1.

We further asked how active and costly integron integrases can be maintained in bacterial populations and explored this with a mathematical model and computer simulations. Our modeling data suggested that continuous environmental fluctuations and episodic selection[29] are prerequisites for effective maintenance of a functional IntI1 over time unless its activity is domesticated by host-mediated LexA repression. However, in our model effective maintenance of integrons with functional integrases depends on continuous

supply of new gene cassettes as well as an immediate selective advantage provided by these genetic elements. The origin, evolution and supply of new gene-cassettes to integron platforms is currently unknown, and needs further exploration for a more complete understanding of integron evolution.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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