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Integron in Gram negative bacteria: Classes and fitness cost

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ABSTRACT

Bacteria intrinsically harbor methods of gene exchange that are distinct from those in eukaryotes, but subject to natural selection laws. Dissemination of antimicrobial resistance is one of the major consequences of gene exchange ability. Bacterial genomes have the ability to undergo evolutionary changes within specified time frame resulting in an exceptional diversity, especially, under strong selective pressure. One of the most important elements of antimicrobial resistance are integrons which serve as a platform for gene cassettes integration and expression.

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INTRODUCTION

Integrons were characterized in the late 1980s as a major contributor to the dissemination of antibiotic resistant determinants among Gram-negative bacteria.[1] Though each has unique features, chromosomal and mobile integrons exist with the latter, as the name implies, carried on mobile elements such as transposons and plasmids and largely associated with antibiotic resistance. The general structure of an integron includes an integrase gene, gene cassettes and a recombination site as well as two conserved regions, one at each end of the integron structure: the 5' and 3' conserved sequences (CS).[2]

Integrons are classified according to their integrase (*intI*) gene sequence. Integrase is a tyrosine recombinase enzyme however; it has been found that integrase differs from the other tyrosine recombinase family members in a certain motif that is unique for integrase and is essential for its activity.[3] It has recently been shown that stress responses such as the SOS response may lead to integrase overexpression, thus increasing the likelihood of gene cassette rearrangement in integrons.[4]

Gene cassettes are usually promoter-less and consisting of an open reading frame and an adjacent recombination site known as 59 be or *attC*, specifically recognized by the integrase enzyme. Multiple gene cassettes assembled into an array could be included in a single integron structure; however, integrons with no gene cassettes have also been observed. The majority of gene cassettes identified are associated with antibiotic resistance. An integron general promoter (Pc)located within the sequence of the *intI* gene directs the expression of all the gene cassettes. Additionally, a second promoter may also exist in some integrons. The strength of expression between the gene cassettes is variable depending on how close each gene cassette is to the promoter region meaning that proximal gene cassettes are expressed more strongly than distal ones. This is believed to be due to transcription attenuation with the transcript span.[5] Other factors that could affect the level of expression include the presence of internal promoters. The 59 be are of variable length between 57-141 bp with 25 bp terminal inverted repeats (Figure 1). This article aims to go through the common classes of integrons, their structure, medical importance and fitness cost.

Class 1 integron

In Class 1 integrons, the 5'-CS includes the *intI1* gene whereas the 3'-CS contains the *qacE* (encoding quaternary ammonium compounds resistance)and *sul1* (encoding sulfonamide resistance)genes in addition to an open reading frame (ORF)with unknown function.[6]

An atypical 3'CS is also observed in some cases, such as the presence of another ORF insertion of *qacE* as

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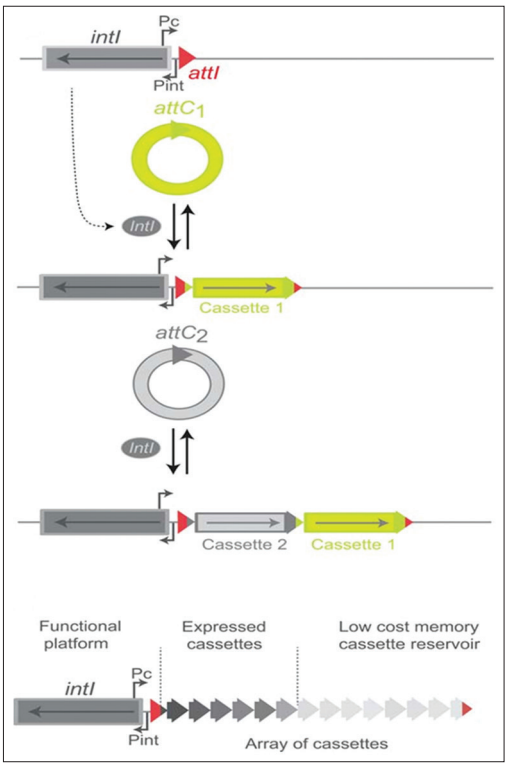

Figure 1: General structure of an integron and the gene cassette (GC) integration/excision mechanism. The integrase enzyme, encoded by intI, catalyzes the insertion and the excision of the gene cassettes. Adapted from Escudero et al.[2]

a gene cassette or deletion of some regions.[7] Integrons themselves are not mobile but they are normally carried and mobilized by transposons. It has been proposed that some integrons are defective transposons, which suggests an evolutionary history of integrons that lost their transposition functions.[8]

Class 2 integron

Class 2 integrons typically have a defective, non-functional integrase encoded by a gene that shares 46% homology with *intI1* commonly found to be associated with the Tn7 transposon family and its derivatives (Tn*1825*, Tn*1826* and Tn*4132*) with its Promoter (Pc) and recombination site attI2 inserted within the transposons.[9] Classically, class 2 integrons contains an array of gene cassettes, including dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (*sat1*), and aminoglycoside adenyltransferase (*aadA1*), confering resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively.[10] Additionally, class 2 integrons share some gene cassettes with class 1 integrons, including *dfrA1, sat1* and *aadA1*.[11]

Class 2 integrons is generally less prevalent than class 1 integron, and have so far been reported in a range of Gram-negative organisms such as *Acinetobacter, Enterobacteriaceae, Salmonella and Psuedomonas*.[12]

It has been shown in *E. coli*, that *intI2* is capable of specific excision and integration of gene cassettes precisely into *attI2*, for instance, erythromycin esterase gene (ereA) was found to be associated with class 2 integron including its own promoter along with an insertion sequence element (IS1)upstream of the *intI2* gene.[13] In *Acinetobacter baumannii*, a novel rearrangement of a class 2 integron with new cassettes in the variable region were identified and found to be different antibiotic resistance genes (*sat2, aadB and catB2*) inserted upstream of the 3 conventional antibiotic resistance genes of *Tn7* class 2 integron.[14]

Class 3 integron

Class 3 integron shares comparable functions to *IntI1* and was found to be associated with soil and freshwater *Proteobacteria* group. This class was first isolated from *Serratia marcescens* strains acquiring bla_{IMP} in Japan in 1993 followed by the identification of *Klebsiella pneumoniae* harbouring bla_{GES-1} gene.[15] This class is less frequent than class 1 and 2 and so far has been identified in a range of Gram Negative species such as *Acinetobacter* spp., *Citrobacter freundii, Alcaligenes Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens* and *Salmonell*a spp.[16]

Class 3 integrons in Gram-negative bacteria was also isolated from hospital sewage containing *Acinetobacter* spp, *Aeromonas* spp and *Citrobacter freundii* with oxacillinase gene cassette as well as cassettes encoding aminoglycoside and β-lactam resistance implicated.[17]

Epidemiology of integrons

Integrons are ubiquitous in nature. Since their discovery, they gained considerable attention, mainly because of their association with the dissemination of antibiotic resistance. The widespread nature of integrons and their stability triggered lots of investigations about their evolution and distribution in diverse environments.[18]

One study aimed to assess the prevalence of class 1 integrons among bacterial isolates from European hospitals in nine countries. The authors demonstrated that 43% of 163 unrelated Gram-negative isolates were integron positive, carrying resistance to aminoglycosides, fluoroquinolones and β-lactams including the third generation cephalosporins and monobactams.[19] Schmitz et al assessed 278 strains representing 11 different Gram-negative species isolated from blood samples for the presence of integrons.[20] Their work showed that 13% of the isolates (belonging to six species)carried integron structures. The increased prevalence of integrons, and particularly of those with several gene cassettes over time is also evident in the clinical settings were the selective pressure is high. Another study by Schmitz et al targeted the prevalence of integrons among enterobacterial

blood-culture isolates, demonstrating that their prevalence elevated from 4.7% in 1993 to 17.4% in 1999.[21] Studies targeting the prevalence of integrons in China indicated a level of 52% among *E. coli* clinical isolates.[22] Another Chinese study conducted 6 years later found that nearly 85% of *E. coli* clinical isolates were integron positive.[22] In the USA, a study estimated the prevalence of class 1 integrons among *E. coli* isolates of human and animal origin and showed that among 274 isolates, 16% were found to carry class 1 integrons.[23]

Several studies have suggested that mobile integrons carrying antibiotic resistance gene cassettes are widely spread among bacterial genomes due to the direct or indirect selective pressure of antibiotic use in clinical and environmental settings.[24,25] Certainly, even in the absence of antibiotic selection, factors such as heavy metals and the presence of quaternary ammonium compounds could also select for the dissemination of mobile integrons. Studying the faecal carriage of *E. coli* in animals, Skurnik et al noticed that the close proximity of humans to animals increases the prevalence of integrons in the animals' bacterial flora.[25]

When Stokes et al examined bacteria isolated from soil and sediments where no obvious antibiotic selection existed, class 1 integrons was identified from 4 different bacterial species not known to be present in humans.[26] None of the 4 integrons isolated had antibiotic resistance gene cassettes in them. The study suggested that evolution and dissemination of integrons may have begun before the antibiotic era. Recently Khosravi *et al* screened 93 *P. aeuroginosa* strains isolated from blood and wound, for the presence of class 1 and 2 integron.[27] Their result revealed the presence of class 1 integron in all of blood isolates and in 95.38% of wound isolates but no class 2 integron was detected.

In China, class 1 and 2 integrons were also identified in 45.8% (54/118)and 19.5% (23/118)of *Pseudomonas aeruginosa* isolates, respectively.[10]

In 2016, Kheiri et al, tested 200 *E. coli* isolates, 136 isolates were multi drug resistance (MDR)collected from chicken, human, cattle and sheep. Class 1 was detected in 38% of human isolates relative to 72% of animal isolates.[28] On the other hand, class 2 was found in 8% and 30% of human and animal isolates respectively.[28]

In another study performed on 164 *E. coli* isolates, class 1 and class 2 integrons and class 3 were found in 78.26%, 76.81% and 26.09% MDR isolates, respectively.[29]

A surveillance study investigated the occurrence of class 3 integron among 587 Gram negative isolates reported the prevalence of class 3 integron to be 0.7%.[30]

Fitness cost of integrons

The evolution of antibiotic resistance imposes a fitness cost, demonstrated as reduced growth rate of the resistant pathogen. fitness cost plays a major role in shaping the level of resistance particularly when the selective pressure is high. Studies have shown that the cost of resistance is highly variable among different strains carrying a wide range of resistant determinant. Since integrons serve as a platform for resistant gene cassettes then the fitness cost of the entire integron structure is dependent on that of every gene cassette carried. Starikova et al demonstrated that the newly acquired class-1 integrons from *Salmonella enterica* serovar Typhimurium and *Acinetobacter baumannii* significantly reduced the host fitness in recipient strain of *Acinetobacter baylyi*.[31] Interestingly, fitness was restored when insertional inactivation of the acquired integron *intI1* was applied.

Similar observation was made by Klus et al who noticed elevated fitness cost in the strain studied (*Acinetobacter baylyi*)suggesting its genetic instability.[32]

CONCLUSION

Horizontal gene transfer is one of the most vital bacterial weapons. Integrons are a professional system by which acquiring and rearrangement of gene cassettes take place. The problem is worse when these integrons are located on a mobile element such as plasmids and transposons. Wide range of Gram negative bacteria where found to harbor these structures with most of the gene cassettes discovered are known to express antibiotics resistance and this has a major clinical implication. Fitness cost of the integron structure is yet another clinically relevant concern especially in this era where Multiple drug resistant isolates are causing epidemics threatening the limited antimicrobial options.

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