ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2005, p. 2512–2514 Vol. 49, No. 6
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.6.2512–2514.2005

Class I Integron with a Group II Intron Detected in an *Escherichia coli* Strain from a Free-Range Reindeer

Marianne Sunde*

Section of Bacteriology, National Veterinary Institute, 0033 Oslo, Norway

Received 25 October 2004/Returned for modification 14 January 2005/Accepted 17 February 2005

An *Escherichia coli* strain, isolated from wild reindeer in a remote mountain area, contained a class 1 integron with two unusual features: a group II intron and a cassette with homology to a superintegron cassette. Alignments indicate that *attC* sites of gene cassettes may be insertion sites for introns.

Integrons play an important role in the development of antibiotic resistance in gram-negative pathogens. Class 1 and class 2 integrons, also referred to as multiresistance integrons (MRIs), have a worldwide distribution and are described from bacteria colonizing humans, animals, and farmed fish (7, 14, 15, 18). The backbone structure of an integron contains a conserved region encoding an integrase (*intI*) and a variable region with integrated gene cassettes (16). A gene cassette usually contains a single open reading frame and a recombination site, the *attC* site (59-base element). The *attC* sites consist of an inverse core site and a core site separated by an intervening palindrome of variable length. The inverse core site is defined as RYYYAAC and the core site as GTTRRRY (R = A or G, Y = C or T) (4, 17).

The ancestors of multiresistance integrons (MRIs) and their resistance genes are presumed to be the superintegrons (SIs) (11, 12). A multitude of gene cassettes is inserted within each SI. The *attC* sites of cassettes within a specific SI show extended homology, whereas *attC* sites of cassettes located within a MRI share little sequence homology.

Gene cassettes are probably ancient structures (10), but their origins and evolution are not clear. It has been proposed that the coding DNA and the *attC* site originally had separate origins and that these elements have been joined through a specific assembly process (10, 13). Recently it has been discussed whether bacterial group II introns might have played a role in this assembly process (1). Group II introns are considered novel genetic elements, first discovered from organelles of plants, fungi, and other lower eucaryotes. Almost all identified bacterial group II introns encode reverse transcriptase open reading frames and are mobile genetic elements able to translocate via RNA intermediates (retro-elements) (2, 3).

The *Escherichia coli* strain investigated, strain 2003-10-702 (hereafter termed *E. coli* 702), was recovered from a fecal sample of a free-ranging reindeer (*Rangifer tarandus tarandus*) from a remote mountain area in mid-Norway (Forelhogna; 4,370 feet above sea level). The reindeers in this area are completely wild living and have no contact with humans. They are never provided feed or medical treatment such as antimicrobial therapy. Fecal samples were collected, as part of the Health Surveillance Program for Cervids in Norway, for microbiological investigation (9). *E. coli* strains were tested for susceptibility to oxytetracycline, chloramphenicol, florfenicol, ampicillin, amoxicillin-clavulanate, ceftiofur, trimethoprim, sulfamethoxazole, streptomycin, gentamicin, neomycin, enrofloxacin, and nalidixic acid (9). Of 42 strains, all from different animals, 10 were resistant to one or more of the tested antimicrobials (9). *E. coli* 702 was resistant to sulfonamides (MIC, >2,048 μg/ml) and streptomycin (MIC, 32 μg/ml). The *sul1* and *intI* genes of class 1 integrons were detected using PCR with primers and conditions previously reported (6). Conjugation experiments and plasmid analysis showed that the integron resided on an approximately 80-kb conjugative plasmid. The variable region of the integron was amplified using a

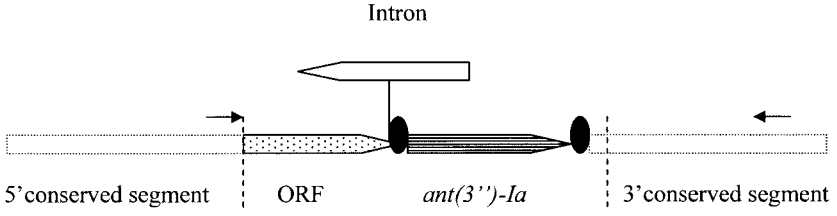

FIG. 1. Organization of the variable region of the class 1 integron reported. The arrows indicate the direction of transcription. The primers used for amplification of the variable region are indicated by small arrows. The two broken vertical lines indicate the region sequenced (shown under accession number AY785243).

* Corresponding author. Mailing address: Section of Bacteriology, National Veterinary Institute, P.O. Box 8156 Dep, N-0033 Oslo, Norway. Phone: 47 23 21 63 81. Fax: 47 23 21 63 01. E-mail: marianne.sunde@vetinst.no.

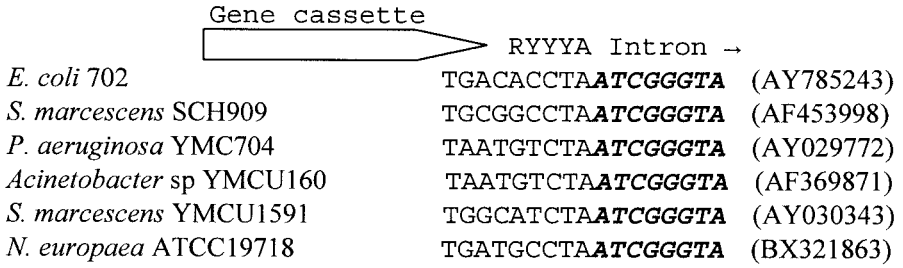

FIG. 2. Alignment of intron insertion points within gene cassettes in class 1 integrons. All introns are inserted to the 5′ end of an *attC* site. The same intron was found in the two upper strains. The same intron, but different from the first, is found in the three next strains. A third intron is present in the *Nitrosomonas europaea* ATCC19718 strain; noncoding DNA is present at the left side of the *attC* site in this strain.

method described previously (7). The primers (5′-TGATGTT ATGGAGCAGCAACGATG-3′ and 5′-CGCACAACCTCG TCGATATCACC-3′) hybridized to the conserved segments of the integron (illustrated in Fig. 1). The sequence of the variable region was subsequently determined. Sequencing was performed on a model 3100-Avant genetic analyzer (Applied Biosystems). Sequences were analyzed using the BioEdit program, the NCBI GeneBlast2 program, and the ClustalW program via the Internet.

The variable region of the integron contained two unusual features: a group II intron and a novel gene cassette (Fig. 1). The *ant(3")-Ia* cassette (5) was also detected, which is one of the most frequently found cassettes in MRIs. The novel gene cassette contained a 402-bp open reading frame of unknown function. The sequence showed 31% identity at protein level to a hypothetical protein encoded by a cassette found in the superintegron of *Xanthomonas* sp. strain CIP 102397 (11) (accession number AF324484).

A 1,970-kb-sized group II intron, encoding a reverse transcriptase-like open reading frame on the complementary strand, was inserted downstream of the reading frame. The intron was equal to an intron found within a class 1 integron in a multiresistant clinical isolate of *Serratia marcescens* (SCH88050909) isolated in 1988 in Greece (1). Alignments showed four nucleotide differences, one of them producing an amino acid alteration (G187S). The intron was identically inserted in *E. coli* 702 and in *S. marcescens* SCH909, between the RYYYA and the AC of an *attC* site, as illustrated in Fig. 2. Another group II intron, distinct from the one reported, has been found within class 1 integrons in a *Pseudomonas aeruginosa* strain (8), in a *S. marcescens* strain (19), and in an *Acinetobacter* sp. strain (20). The group II intron was identically inserted in all three strains, in the same way as reported here (illustrated in Fig. 2). The presence of group II introns, inserted identically at the 5′ end of *attC* sites of gene cassettes, might indicate that this site is a preferred insertion site for such genetic elements.

A gene cassette from the SI of a *Vibrio fischeri* strain contains a 56-codon segment with homology to intron maturase (13) (accession number AY177199, cassette c667-2). This indicates that introns can be involved with cassettes in SIs as well.

It is suggested that some environmental organisms, like *Nitrosomonas europaea*, can serve as reservoirs of integron components since these species contain integrase-like genes and *attC* sites not associated with a gene cassette (1). An *attC* site located adjacent to a group II intron in an *N. europaea* strain (Acc no BX321863) differs by only one nucleotide when aligned with the *attC* of *E. coli* 702 (Fig. 3). This may be a further indication of a connection between environmental bacteria, introns and integron components.

The intron of *E. coli* 702 was still present at the *attC* site in both the isolated strain and its transconjugant, following 3 months of continuous culture in nutrient broth at room temperature (recultured every fortnight). Further investigations will soon be carried out in our laboratory to investigate possible intron mobility to *attC* sites of gene cassettes.

Most MRIs so far characterized originate from bacteria isolated from environments where antibiotics are heavily used. This study shows that class 1 integrons can be found in bacteria occurring in "antibiotic-free" environments, located far away from human activities. This may indicate that class 1 integrons are more prevalent in nature than originally thought and that they have existed since before the antibiotic era.

During the last 15 years, since the discovery of the integron structure, hundreds of integrons have been investigated. Integron-borne group II introns have been detected only five times. Most integrons characterized to date originate from clinical isolates subjected to antibiotic selection force. The search for integrons in environments with no history of antibiotic exposure could perhaps lead to further findings of group II introns, and hopefully their role could be fully determined.

Nucleotide sequence accession number. The sequence of the variable region of the integron was submitted to GenBank under accession number AY785243.

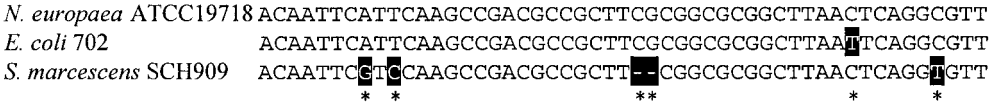

FIG. 3. Alignment of *attC* sites associated with introns in *E. coli* 702, *S. marcescens* SCH909, and *N. europaea* ATCC19718. The same intron was inserted adjacent to the *attC* sites in *E. coli* and *S. marcescens*, whereas a different intron was found in the *N. europaea* strain.

Atle Lillehaug (National Veterinary Institute), Henning Sørum, and Jostein Bjorland (The Norwegian School of Veterinary Science) are acknowledged for collaboration and constructive comments on this paper.

This work was supported by a grant (153080/120) from the Norwegian Research Council.

REFERENCES

1. **Centrón, D., and P. H. Roy.** 2002. Presence of a group II intron in a multiresistant *Serratia marcescens* strain that harbors three integrons and a novel gene fusion. Antimicrob. Agents Chemother. **46:**1402–1409.
2. **Cousineau, B., S. Lawrence, D. Smith, and M. Belfort.** 2000. Retrotransposition of a bacterial group II intron. Nature **404:**1018–1021.
3. **Dai, L., and S. Zimmerly.** 2002. Compilation and analysis of group II intron insertions in bacterial genomes: evidence for retroelement behavior. Nucleic Acids Res. **30:**1091–1102.
4. **Hall, R. M., D. E. Brookes, and H. W. Stokes.** 1991. Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination cross-over point. Mol. Microbiol. **5:**1941–1959.
5. **Hollingshead, S., and D. Vapnek.** 1985. Nucleotide sequence analysis of a gene encoding a streptomycin/spectinomycin adenyltransferase. Plasmid **13:** 17–30.
6. **Kerrn, M. B., T. Klemmensen, N. Frimodt-Møller, and F. Espersen.** 2002. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. J. Antimicrob. Chemother. **50:**513–516.
7. **L'Abèe-Lund, T. M., and H. Sørum.** 2001. Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. Microb. Drug Resist. **7:**253–262.
8. **Lee, K., J. B. Lim, J. H. Yum, D. Yong, Y. Chong, J. M. Kim, and D. M. Livermore.** 2002. bla_{VIM-2} cassette-containing novel integrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. Antimicrob. Agents Chemother. **46:**1053–1058.
9. **Lillehaug, A., B. Bergsjø, J. Schau, T. Bruheim, T. Vikøren, and K. Handeland.** 2005. *Campylobacter* spp., *Salmonella* spp., verocytotoxic *Escherichia coli*, and antibiotic resistance in indicator organisms in wild cervids. Acta Vet. Scand. **46:**23–32.
10. **Recchia, G. D., and R. M. Hall.** 1997. Origins of the mobile gene cassettes found in integrons. Trends Microbiol. **5:**389–394.
11. **Rowe-Magnus, D. A., A.-M. Guerout, P. Ploncard, B. Dychinco, J. Davies, and D. Mazel.** 2001. The evolutionary history of chromosomal super-integrons provides an ancestry for multi-resistant integrons. Proc. Natl. Acad. Sci. USA **98:**652–657.
12. **Rowe-Magnus, D. A., A.-M. Guerout, and D. Mazel.** 2002. Bacterial resistance evolution by recruitment of super-integron gene cassettes. Mol. Microbiol. **43:**1657–1669.
13. **Rowe-Magnus, D. A., A.-M. Guerout, L. Biskri, P. Bouige, and D. Mazel.** 2003. Comparative analysis of superintegrons: engineering extensive genetic diversity in the Vibrionaceae. Genome Res. **13:**428–442.
14. **Sallen, B., A. Rajoharison, S. Desvarenne, and C. Mabilat.** 1995. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of *Enterobacteriaceae*. Microb. Drug Resist. **1:**195–202.
15. **Sandvang, D., F. M. Aarestrup, and L. B. Jensen.** 1998. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. **160:**37–41.
16. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
17. **Stokes, H. W., D. B. O' Gorman, G. D. Recchia, M. Parsekhian, and R. M. Hall.** 1997. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. Mol. Microbiol. **26:**731–745.
18. **Sunde, M., and H. Sørum.** 1999. Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. Microb. Drug Resist. **5:**279–287.
19. **Yum, J. H., D. Yong, K. Lee, H. S. Kim, and Y. Chong.** 2002. A new integron carrying VIM-2 metallo-β-lactamase gene cassette in a *Serratia marcescens* isolate. Diagn. Microbiol. Infect. Dis. **42:**217–219.
20. **Yum, J. H., K. Yi, H. Lee, D. Yong, K. Lee, J. M. Kim, G. M. Rossolini, and Y. Chong.** 2002. Molecular characterization of metallo-β-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new integrons carrying the bla_{VIM-2} gene cassettes. J. Antimicrob. Chemother. **49:**837–840.