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 I 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 transpose 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.

---

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.

2512
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 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.

![Gene cassette](image)

**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.

![Gene cassette](image)

**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.
REFERENCES


