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The Potential of Integrons and Connected Programmed Rearrangements for Mediating Horizontal Gene Transfer

LARS SUNDSTRÖM

Pharmaceutical Biosciences, Microbiology Division
P.O. Box 581 BMC, S-75123 Uppsala, Sweden

SUMMARY

Site-specific recombination of integrons, mediates transfer of single genes in small genomes and plasmids. Recent data suggest that new genes are recruited to the cassettes – the units moved by integrons. Integrons are resident in a class of transposons with pronounced target selectivity for resolution loci in broad host range plasmids. A resulting network of programmed transfer routes, with potential offshoots reaching into eukaryotic cells, may channel genes to unexpectedly remote organisms. It has previously been observed that the conjugation apparatus of the broad host range plasmid R751 (IncP) which contains transposon Tn*5090* harbouring an integron, promotes horizontal genetic transfer between bacteria and yeast. Furthemore, it is well known and fundamental for widely used gene replacement technologies, that site-specific recombination systems (e.g. Cre-*lox* of bacteriophage P1) related to the integrons are functional in higher eukaryotes. It seems very clear that integrons and associated programmed transfer mechanisms have high significance for the dissemination of antibiotic resistance genes in bacteria wheres further studies are needed to assess their importance for spreading of arbitrary genes in a wider range of host systems.

TEXT

Highly organized transfer of monogenic modules called cassettes allows acquisition and clustering of individual genes in bacterial genomes and plasmids. A large number of antibiotic resistance gene combinations have been formed in plasmids due to integration of different cassettes in tandem. In this text will be discussed the potential recruitment of new genes to the cassettes and horizontal genetic transfer of cassettes between widely different organisms.

The movement of a gene from one location to another requires cleavage and rejoining of DNA strands, a process called genetic recombination. Two forms of specialized recombination, site-specific recombination and transposition, have different roles in the transfer of genes (Craig, 1988; Nash, 1996: Hallet and Sherratt, 1997). Site-specific recombination elements, integrons (Ouellette and Roy, 1987; Sundström *et al.*, 1988; Stokes and Hall, 1989; Recchia *et al.*, 1995; Hansson *et al.*, 1997), mediate the organized transfer of genes borne on cassettes. Transposons, on the other hand, account for a connection between integrons carrying various selections of cassettes with horizontal transfer mechanisms (Rådström *et al.*, 1994). While most other transposons are inserted more or less randomly those found to harbour integrons are highly selective with respect to their target sites (Kamali-M., and Sundström, unpublished).

Integrons were first identified by the great variation of genes precisely inserted in a conserved context on resistance plasmids of gram negative bacteria (Sundström and Sköld, 1986; Cameron *et al.*, 1986; Ouellette *et al.*, 1987; Sundström *et al.*, 1988). The integrons carry their cassettes just

downstream of a recombinase-encoding, conserved sequence (CS) containing a strong promoter (Levesque *et al.*, 1994). The promoter is used for expression of the inserted cassettes but has potential significance also for regulating the frequency of recombination. The cassettes carry a wide spectrum of genes several of which mediate antibiotic resistance (Huovinen *et al.*, 1995; Recchia and Hall, 1995). Resistance to antibiotics such as e.g. aminoglycosides, trimethoprim, and beta-lactams are represented.

Site-specific recombination is conservative process which requires neither replication nor ATP hydrolysis (Craig, 1988; Nash, 1996). It uses a hydroxyl nucleophile inherent to the recombinase polypeptide and the catalysis is independent of metal ions. The site-specific recombinases of integrons (IntI proteins) mediate a low level of recombination between integron cassette sites *in vitro* (Gullberg *et al.*, unpublished). The IntI proteins belong to the same protein family as the integrase used for integrative lysogenization of bacteriophage lambda. All these recombinases use the hydroxyl group in a tyrosine as nucleophilic reagent. The chromosome dimer resolvase Cre of phage P1 is another well-known member of the tyrosine recombinase class (Argos *et al.*, 1986; Esposito *et al.*, 1997; Guo *et al.*, 1997; Yang and Mizuuchi, 1997). The biochemical steps of site-specific recombination mediated by tyrosine recombinases are assumed to follow a common route with minor degree of heterogeneity among the different mechanisms.

Site-specific recombination commonly occurs within a limited makeup of sites while the integrons, by contrast, select the partner sites from a larger pool. A resulting combinatorial effect on the outcome is displayed as various antibiotic resistance patterns. The complexity of sites of other site-specific recombinases range from simple arrangements of two spaced inverted repeats (for instance *lox*P of the P1 Cre recombinase; Hoess and Abremski, 1984) to complicated sites promoting the formation of higher order nucleoprotein complexes (e.g. *att*P of the lambda integrase; Landy, 1989). The high complexity of many recombination sites is related to a need for regulation or site orientation selectivity (Baker and Mizuuchi, 1992; Echols, 1990; Grindley, 1993; Rice, 1997). Site-specific recombinations create either deletions/insertions or inversions depending on the disposition of the two partner sites.

The function and organization of the sites used for gene-transfer between integrons cassettes were recently studied (Francia *et al.*, 1997; Hansson *et al.*, 1997; Hansson and Sundström, submitted; Stokes *et al.*, 1997). When cassettes are integrated in tandem a site called *attC* is formed between them (Fig. 1A). In the removal or inclusion of cassettes in integrons these sites must be involved.

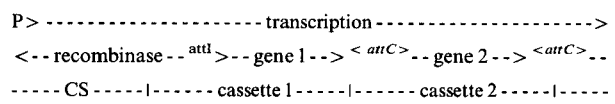

Fig. 1A. The organization of an integron.

The *attC* sites show conservation only close to the ends (Sundström *et al.*, 1993), but show long dyad symmetries over an area of at least 50 bp (Cameron *et al.*, 1986; Martinez and de la Cruz, 1990). The *attC* site which occurs downstream of the streptomycin resistance gene *aadA1* in Tn*21* comprises approximately 65 bp. It carries a short core containing the cross-over point on the right end and an extended arm region beginning with a core-complementary sequence on the left end (Fig. 1B). A consensus based on the aligned sequences of several *attC* sites and mapped secondary cross-over loci inside them indicated two subsites (Francia *et al.*, 1997; Stokes *et al.*, 1997; Hansson and Sundström, 1998). The left and right subsites which closely resemble each other were arranged similar to sites of other site-specific recombinases. Organization of a recombination site into multiple subsites is likely to be related to the assembly of a higher order nucleoprotein complex (Grind-

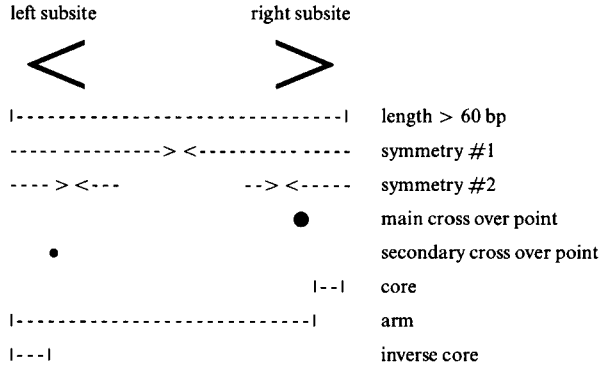

Fig. 1B The organization of *att*C.

ley, 1993). The inverse core in integron cassettes, which rarely recombines itself, was highly responsive to mutations. This stimulation of recombination suggests that the inverse core is a key sequence in the formation of a higher order molecule complex (Hansson and Sundström, 1998).

Cassettes have been observed to integrate with preference for another type of site, *attI*, which occurs once per integron at the end of the conserved sequence and close to a strong promoter (Levesque *et al.*, 1994; Recchia *et al.*, 1994; Hansson *et al.*, 1997). Recombination with secondary sites are rare as compared to recombination between primary sites such as *attC* or *attI* (Francia *et al.*, 1993; Recchia *et al.*, 1994). The secondary sites include the short sequence GNT but site recognition seems to require further sequences, such as a symmetric counter element (Francia *et al.*, 1997). Recombination between all combinations of *attC*, *attI* and secondary sites have been documented (Martinez and de la Cruz, 1990; Francia *et al.*, 1993; Recchia *et al.*, 1994; Hansson *et al.*, 1997). Although recombination between two secondary sites has been indicated only by sequence observations such events could have significance for moving the integrase gene to new locations or for the creation of new cassettes.

The principal cross-over point in *attC* is located in the core (Fig. 1B) on the right end of the site while less frequent recombination occurs at the left end (in the inverse core). The integrons seem to use the power and precision in the site-specific recombination mechanism itself for fusing *attC* by the left hand cross-over site, to upstream secondary sites (Fig. 1C). A fraction of translation stop codons (TAA) seem to coincide with secondary sites which could increase the likelyhood for connection between a gene and an *attC*. The addition of a recombinative handle (*attC*) on the 3'end a gene is probably a crucial step in the formation of a cassette. A cassette that is available for integration may be represented as a minicircle formed by site-specific excision (Collis and Hall, 1992a and b). The connection of closely related *attC* sites to unrelated genes in integrons supports that these have been acquired and that a mechanism for fusion of *attC* sites to genes operates (Recchia and Hall, 1995). Very few genes that are either borne or not borne on cassettes have been described. Therefore, it is not known to what extent resident genes are recruited to the cassettes. This can only be understood by further study of cassettes on mechanistic and genomic level.

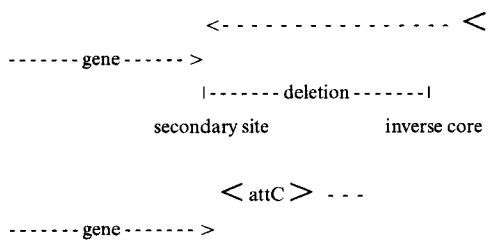

Fig. 1C. Model for the formation of a pro-cassette.

Three classes of integrons have been observed on plasmids (Cameron *et al.*, 1986; Ouellette *et al.*, 1987; Sundström *et al.* 1988; Stokes *et al.*, 1989; Martinez and de la Cruz, 1990; Arakawa *et al.*, 1995; Recchia and Hall, 1995; Hansson *et al.*, submitted) and a fourth class was recently described in the chromosome of *Vibrio cholerae* (Mazel *et al.*, 1998). The amino acid sequences of the integrase genes of the four classes differ to about 50%. The specificity of the recombinases for sites of the own class seems to be relaxed and cassettes have been documented in multiple classes of integrons (Sundström and Sköld, 1990; Arakawa *et al.*, 1995). It is possible that the plasmid-borne integrons originate from chromosomal integrons in certain bacteria. The *attC* sites of different cassettes inserted in plasmids are strikingly heterogeneous sequences. By contrast, *attC* sites on chromosomal cassettes in *Vibrio cholerae* are highly conserved (Barker *et al.*, 1994; Clark *et al.*, 1997; Mazel *et al.*, 1998). It is likely that the *attC* sites form a conserved repeat family in the genome of a defined bacterial species whereas the *attC* sites in two organisms might differ. Horizontally transferred integrons are likely to have acquired *attC* sites from various hosts and it follows that the sequence variation among those sites could be higher than among sites confined to one microrganism.

Transposons have linked the integrons to horizontally transferred replicons. Both class 1 and class 2 integrons are borne on transposable elements related to the transposing bacteriophage, Mu (Tn*5090* and Tn7, respectively; Barth *et al.*, 1976; Shapiro and Sporn, 1977; Craig, 1991; Rådström *et al.*, 1994). Mu uses transpositional recombination both for establishing lysogeny in its host,

E. coli, and for replication under vegetative phase. To be efficient, Mu transposition requires two proteins, a transposase (A) and an ATP-binding protein (B). The assembly of the A protein into an active tetrameric subunit holoenzyme occurs by contacts with 20-meric iterons on both transposon ends (Baker and Mizuuchi, 1992). Proteins related to proteins A and B are encoded by both Tn*7* and Tn*5090* whereas most other transposons do not code for a B protein. Both Tn*7* and Tn*5090* furthermore carry iterons for transposase binding on the ends similar to those on Mu ends (Rådström *et al.*, 1994). Transposons in general, including Mu, are more or less blind with respect to the target site but Tn*7* and Tn*5090* both show marked target preference. For instance, Tn*7* transposes with high preference for a unique site in the chromosomes of a range of different bacteria and shows secondary preference for conjugative plasmids (Craig, 1997). Tn*5090* shows marked insertion preference for regions close to resolvase genes in broad host range plasmids of IncP and IncW (Kamali-M. and Sundström, unpublished). The *par* region in IncP plasmid RP1 is a frequently used target which in the context of horizontal gene spreading is interesting because the homing of a transposon links a conjugative mechanism to transfer of single genes. The mother plasmid (R751 of IncP) of the integron-carrying transposon Tn*5090* is closely related to RP1 and has been reported to mediate conjugation of DNA between bacteria and yeast (Heinemann and Sprague, 1989). The conjugative apparatus is entirely plasmid coded (Heinemann and Ankenbauer, 1993).

Some site-specific recombination mechanisms related to that used for movement of integron cassettes occurs efficiently in a wide range of cells, eukaryotic cells as well as bacteria. One example is the originally bacteriophage-based Cre-*lox* system which is a widely used tool in knock-out technology in part because it functions efficiently without bacterial host factors and also in mammalian cells (Sauer and Henderson, 1988). It thus seems reasonable to speculate that transfer of integron cassettes may in theory occur in any cell and that there is a connection to horizontal transfer between bacteria and eukaryotic cells. A potential transfer chain for arbitrary genes seems plausible.

In conclusion, integrons are documented to be important for the transmission of genes through bacterial populations by loading different sets of genes onto the appropriate vehicles. New evidence support that the integrons are also capable of recruiting resident genes to the cassettes and thus endow them with a capacity for being spread efficiently. The integrons are borne on certain transposons preferently inserted into conjugative plasmids and some of these have a remarkably wide host range. In addition to transfer between widely different bacteria these broad-host range vectors have furthermore been reported to mediate transfer between bacteria and yeast. However, further evidence that the integrons have indeed contributed to horizontal gene spreading between widely different biological systems is needed.

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