Horizontal transfer of genes in bacteria

Paul H. Roy

Horizontal transfer of genes in bacteria has been studied for several years, especially with regard to extrachromosomal elements. Genomic sequencing is now providing increasing evidence for widespread exchange of chromosomal genes. I will attempt to provide a brief overview of the mechanisms of genetic exchange and of mobile DNA, and then summarize how genomic data can be searched for evidence of horizontal transfer.

● Mechanisms of genetic exchange

Conjugation. Conjugation has been well studied, especially in *Escherichia coli* and *Pseudomonas aeruginosa*. In these species, sex factor (F and FP) plasmids can become integrated into the chromosome by homologous recombination between an insertion sequence (IS) on the plasmid and a homologous IS on the chromosome. Subsequent pilus synthesis and initiation of single-strand displacement replication then causes the transfer of half of the F plasmid followed by a large segment of chromosomal DNA, via the pilus, into a recipient cell. There, the second strand is synthesized and the acquired DNA is incorporated by homologous recombination. Alternatively, the F factor can be aberrantly excised to form an F′ factor (a large circular plasmid containing many chromosomal genes) which can be transferred by the same mechanism. Because of the host range of the F plasmid, transfer between *E. coli* and *Salmonella* can occur. Amongst Gram-positive organisms, streptococci are the best studied and contain both conjugative plasmids and conjugative transposons (see below).

Transformation. This mechanism, while not ubiquitous, has been well studied in both Gram-positive (*Streptococcus pneumoniae* and *Bacillus subtilis*) and Gram-negative (*Haemophilus influenzae* and *Neisseria gonorrhoeae*) bacteria. Several other species are known to have natural transformation systems, among them *Acinetobacter* (where transformation may be the major mechanism of dissemination of antibiotic resistance in this species) and the cyanobacterium *Synechocystis*. The completion of the genomic sequences of *B. subtilis* and *H. influenzae* and the identification of their competence genes makes it possible to search for homologues in other species.

Transduction. Two types of transduction exist: specialized (by aberrant excision of a lysogenic phage, incorporating genes adjacent to the phage attachment site) and generalized (by erroneous encapsidation of chromosomal DNA in lieu of linear phage concatameric DNA produced by rolling circle replication). The specificity is restrained by the phage host range; this is probably not a major mechanism of interspecies transfer.
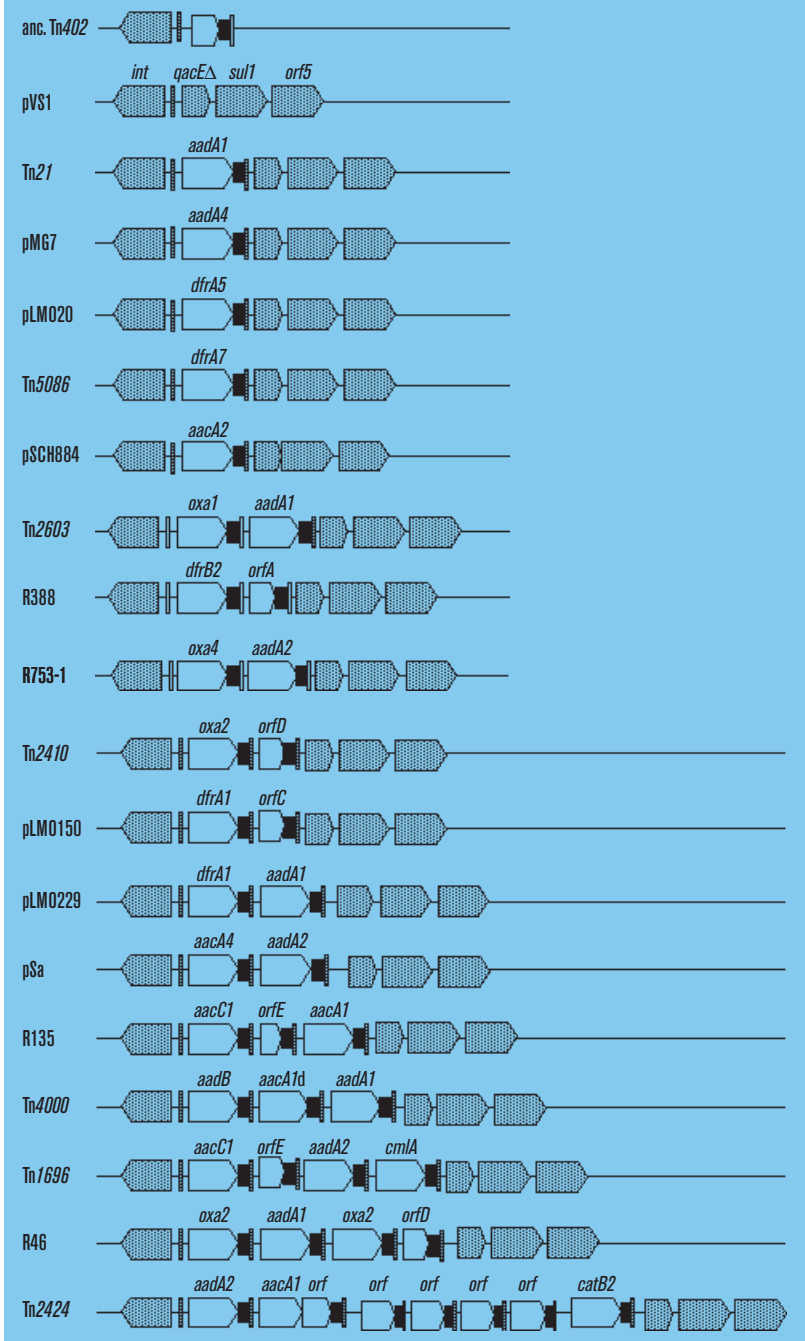
● Vehicles for genetic exchange

Plasmids. Plasmids have been best studied in *E. coli* and *P. aeruginosa*. Early on they were divided into F (sex factor) and R (resistance) plasmids. Later, many R plasmids were found to encode F-like replication and/or transfer genes. Conjugative R plasmids are responsible for most of the dissemination of antibiotic resistance genes. One of the best studied is R100, which contains F-like transfer genes but different replication genes. Within R100 there is a transposon, Tn*10*, encoding tetracycline resistance, and another transposon, Tn*21*, encoding mercury resistance, itself within a Tn*9*-like transposon encoding chloramphenicol resistance. Within Tn*21* there is an integron encoding streptomycin and sulphonamide resistance. While the aforementioned are typically extrachromosomal genes, plasmids can also carry chromosomal genes. A good example is the class C chromosomal β-lactamases, whose genes are increasingly found on plasmids. As mentioned above, F plasmids can transfer large blocks of chromosomal genes.

Transposons. Transposons, or 'jumping genes' are responsible for the dissemination of several antibiotic and heavy metal resistance genes, as well as degradative genes and even the lactose operon (the latter with the consequence that clinical microbiologists can no longer ignore Lac$^+$ enterobacteria!). Perhaps the best-known example is Tn*3*, which contains two genes for its own transposition and the TEM-1 β-lactamase gene. Tn*3* has spread among *Enterobacteriaceae* on plasmids of several incompatibility groups (which correspond to replication functions and thus to host ranges). But perhaps the best example of horizontal transfer by transposons is the arrival of Tn*3* in *Haemophilus* and *Neisseria* in the mid-1970s. Although not all the pieces of the puzzle can be retraced, the most likely scenario would involve the transfer, by conjugation or transformation, of an *E. coli* plasmid carrying Tn*3* into *Haemophilus ducreyi* (the agent of soft chancre). The plasmid would not have been able to replicate (*E. coli* plasmids are unknown in *Haemophilus*) but before it was degraded, its Tn*3* 'hopped' onto a native plasmid. The *H. ducreyi* plasmids are the 'smoking gun' since they contain all of Tn*3*. A 'streamlined' version, having lost the transposase gene (no longer necessary since the plasmids are spread among *Haemophilus* and *Neisseria* by transformation), is found not only in *H. ducreyi* but also in *Haemophilus parainfluenzae* and *N. gonorrhoeae*, where it caused the emergence of the well known penicillinase-producing *N. gonorrhoeae* (PPNG). Surprisingly, the TEM gene in these organisms has not produced the wide variety of extended-spectrum TEM β-lactamases found in *Enterobacteriaceae* despite the widespread use of ceftriaxone to treat PPNG – does *N. gonorrhoeae* have a very faithful DNA polymerase?

Complex transposons, such as Tn*5*, are composed of a central section, typically containing one or more antibiotic resistance genes, flanked by inverted or direct repeats of ISs. These transposons are probably formed by

anc. Tn*402*

int *qacE*Δ *sul1* *orf5*
pVS1

aadA1
Tn*21*

aadA4
pMG7

dfrA5
pLM020

dfrA7
Tn*5086*

aacA2
pSCH884

oxa1 *aadA1*
Tn*2603*

dfrB2 *orfA*
R388

oxa4 *aadA2*
R753-1

oxa2 *orfD*
Tn*2410*

dfrA1 *orfC*
pLM0150

dfrA1 *aadA1*
pLM0229

aacA4 *aadA2*
pSa

aacC1 *orfE* *aacA1*
R135

aadB *aacA1d* *aadA1*
Tn*4000*

aacC1 *orfE* *aadA2* *cmlA*
Tn*1696*

oxa2 *aadA1* *oxa2* *orfD*
R46

aadA2 *aacA1* *orf* *orf* *orf* *orf* *orf* *catB2*
Tn*2424*

Integrons are elements in which structural genes, each linked to a palindromic '59-base element' (*attC* site), are assembled as cassettes into operons by insertion at an attachment (*attI*) site. A strong promoter adjacent to the *attI* site makes integrons a sort of natural expression vector. In class 1 integrons, the most frequent kind, the integrase and *attI* site (from a cryptic phage in chromosomal DNA?) became associated with a Tn*5053*-like transposon, and a few have retained this association, e.g. Tn*402* of plasmid R751. Similarly, class 2 integrons became associated with an ancestral transposon to form Tn7, whose integron carries its resistance genes. Most elements carrying class 1 integrons are descended from an element which acquired sulfonamide resistance (in the 1930s?), but in turn lost two of the four transposition genes. However, one of these was able to regain mobility by inserting into a mercury resistance transposon, Tn*2613*, to form Tn*21*. Many different cassette arrangements are found in Tn*21*-like transposons.

Although no direct chromosomal ancestor of class 1 or 2 integrons has yet been found, genomic sequencing has revealed several new classes of integrons, indicating that they are ancient mechanisms of gene exchange which have only recently been co-opted for dissemination of antibiotic resistance. In *Vibrio cholerae* there is a 'super-integron' in which over 150 cassettes (mostly unidentified genes), each with a palindromic 'VCR repeat' (a variant *attC* site) are aligned in a row next to an integrase gene. Other classes of integrons exist in *Xanthomonas campestris* pv. *badrii*, *Shewanella putrefaciens*, and in *Nitrosomonas europaea*. The *attC* sites in their cassettes are much more similar to the '59-base elements' of class 1 integrons than are VCR repeats. The integrases of the various classes, however, all show 45–60 % amino acid identity between themselves.

The arrangement of some representative integrons is shown in Fig. 1.

transposition of ISs to either side of a region of DNA. Indeed, any region of chromosomal DNA which becomes flanked by copies of the same IS can potentially become a transposon. This should be looked for in sequence analysis of chromosomal DNA near putative IS elements.

Conjugative transposons are found in Gram-positive bacteria and encode the mechanism, yet to be described in detail, for their own transfer. They encode an integrase and an excisase related to those of temperate phages. Among these are Tn*916*, encoding tetracycline resistance, Tn*1545*, encoding erythromycin resistance, and Tn*1547*, encoding one type of vancomycin resistance (another is carried on Tn*1546*, a Tn*3*-type transposon).

Integrons. Much of the dissemination of antibiotic resistance in Gram-negative bacteria is due to integrons. Evidence for their existence was hinted at by restriction maps and heteroduplexes, demonstrated by electron microscopy, which pointed to gene-sized differences among related plasmids and transposons. Sequencing confirmed that a mechanism of site-specific recombination, mediated by an integrase, was at work.

Further reading

Jain, R., Rivera, M.C. & Lake, J.A. (1999). Horizontal gene transfer among genomes: the complexity hypothesis. *Proc Natl Acad Sci USA* 96, 3801–3806.

Recchia, G.D. & Hall, R.M. (1995). Gene cassettes: a new class of mobile element. *Microbiology* 141, 3015–3027.

Roy, P.H. (1999). Transposons and integrons: Natural genetic engineering of antibiotic resistance. *Can J Infect Dis* 10, suppl. C, 4C–8C.

● Evidence from sequence data for horizontally transferred genes

G+C anomalies. Even before the sequencing of complete genomes, it was possible to identify certain chromosomal genes as having an origin different from that of other genes from the same organism. Sometimes this can be done when relatively few genes have been sequenced. An example is the gene encoding the BRO-1 β-lactamase of *Moraxella catarrhalis* (which causes otitis media; this species went from <10 to >80 % penicillin-resistant in a few years). The *bro1* gene shows an abrupt shift in G+C content relative to its flanking sequences. This gene, apparently of Gram-positive origin, substituted its ~1000 bp for a 75-bp, mostly intergenic, region found in penicillin-sensitive strains, and in so doing became the third gene of a four-gene operon. The surrounding ORFs show strong similarity to *gatCAB*, encoding glutamyl-tRNA^{Gln} amidotransferase (AdT), usually associated with Gram-positive bacteria. It might appear at first that the whole region is of Gram-positive origin. However, the recently completed genomes of *P. aeruginosa* and *Neisseria meningitidis*, Gram-negative bacteria in which AdT is present and asparaginyl-tRNA synthetase is absent, point to another role for this enzyme – asparaginyl-tRNA^{Asn} amidotransferase. The ORFs would thus be essential genes native to *M. catarrhalis*.

Codon usage. Not all G+C anomalies are related to horizontal transfer; the two anomalous regions in the *H. influenzae* genome correspond to the rRNA operon and to a cryptic phage. When as few as 10–20 genes have been sequenced from an organism, it is possible to construct a codon usage table, which reflects G+C content and also the relative abundance of tRNAs in an organism. Genes, especially those for highly expressed proteins, tend to conform to the codon usage patterns. Exceptions can indicate recent acquisition by horizontal transfer. Examples are the PAK and PAO pilin genes of *P. aeruginosa*. Their anomalous codon usage indicates that their genes have not evolved over a long period in these species.

Phylogenetic tree anomalies. A high degree of similarity of a gene with that of a genetically distant organism may be an indication of horizontal transfer. Similarly, phylogenetic trees for genes common to several sequenced genomes may show anomalies when compared to the rRNA tree. This is less common with informational genes (e.g. those involved with transcription and translation) than with other genes (there are some interesting exceptions, e.g. glutaminyl-tRNA synthetase genes). The rates of evolution for the two groups are similar and the anomalies in phylogenetic trees made with single genes other than informational genes provides evidence for continual events of horizontal transfer during the evolution of prokaryotes.

● Conclusions

Antibiotic use creates a heavy selective pressure which permits us to observe, practically in real time, the evolution of bacteria using pre-existing mechanisms which, before the antibiotic era, were used for exchange of other sorts of genes. This rapid evolution has made extensive use of extrachromosomal elements, but evidence for longer-term exchange at the level of the chromosome can be detected by genomic sequence analysis.

● *Dr Paul H. Roy is a Professor in the Département de Biochimie et de Microbiologie, Université Laval, Ste.-Foy, Québec G1K 7P4, Canada*
Tel. +1 418 654 2705; Fax +1 418 654 2715
e-mail paul.h.roy@crchul.ulaval.ca