Leading articles

Evolution and spread of tetracycline resistance determinants

For more than 40 years the tetracycline group of antibiotics has been a cornerstone of treatment of infectious diseases caused by a variety of bacterial and rickettsial disease agents. Their broad spectrum of activity and relative safety have placed them second only to the penicillins as agents commonly used to treat infections throughout the world. Each year an estimated 10 million pounds of tetracyclines are available worldwide (Col & O'Connor, 1987).

Over the past several decades, however, resistance to these antimicrobials has emerged as an important limitation on their efficacy in the treatment of infections caused by many different aerobic and anaerobic genera (Levy, 1984). Of considerable clinical importance has been the appearance of tetracycline resistance among common disease agents of the respiratory and genitourinary tracts (Roberts, 1989). As the genetic determinants of these resistant organisms have been elucidated, it has become clear that the same or very similar genes are responsible for resistance in a large number of different genera.

More than a dozen distinguishable tetracycline resistance determinants have been described (Levy, 1984, 1988). However, most of these determinants can be grouped into three major families based on relatedness of the DNA sequence and the amino acid sequence of the products encoded. Members of each family presumably evolved from an ancestral determinant. Two of these families code for an active efflux system which keeps intracellular drug concentrations below levels that inhibit protein synthesis. The third family involves determinants that specify ribosomal protection. The DNA sequence of other newly discovered determinants has yet to be determined. Some of these may join the three families already identified, while others may represent new ones. The discovery in tetracycline-producing Streptomyces of genes for both decreased tetracycline uptake and ribosome protection (Ohnuki et al., 1985; Reynes et al., 1988) suggests that this genus may be an ancestral source for some of these determinants.

Tetracycline resistance determinants are often on plasmids and transposons (Levy, 1984). The first such determinant recognized on a transferable plasmid was that of R100 (R222), of historic interest in the discovery of R factors in Japan. The determinant was later shown to reside on transposon Tn10 (Kleckner et al., 1975), one of many now recognized antibiotic resistance transposons. The determinant on Tn10 has both a structural and a repressor gene, which are expressed divergently from overlapping operator regions (Bertrand et al., 1983; Hillen & Schollmeier, 1983). This same genetic organization is found for other members of this family of efflux determinants now designated as Classes A–E, distinguishable by DNA:DNA hybridization, but specifying highly similar products: Classes A–E repressors, 43–63% amino acid identity; Classes A–C structural gene products, 45–78% identity (Nguyen, Postle & Bertrand, 1983; Waters et al., 1983; Unger, Klock & Hillen, 1984). On the basis of these similarities, Classes A and C represent one branch of this family; Classes B (Tn10) and D another, and E possibly a third (Unger et al., 1984; Tovar, Ernst & Hillen, 1988).

Using DNA fragments specific for Classes A–E as probes, we and others have identified a large and ever increasing number of Gram-negative genera, in particular Enterobacteriaceae, which harbour these determinants (Levy, 1984, 1988). Some genera are more likely to have a particular determinant; e.g. tetracycline resistance is commonly specified by Tn10-like determinants in Escherichia coli and Haemophilus spp. (Marshall et al., 1984) while the Class E determinant is common in Aeromonas (DePaola et al., 1988).

A second family of determinants represented by Classes K and L also mediates drug efflux (McMurry et al., 1987) and shares about 65% amino acid sequence homology (Ishida & Shibahara, 1985). These determinants have so far been detected only in the Gram-positive aerobic organisms. The Class K determinant described initially on plasmid pT181 (Khan & Novick, 1983) is common among staphylococci, while the Class L determinant (Burdett, Inamine & Rajagopalan, 1982) is found among streptococci, staphylococci and Bacillus spp.
A third family of determinants, represented by Classes M and O, shows 76% DNA sequence homology (Sougakoff et al., 1987) and specifies resistance by a cytoplasmic factor which protects the ribosome from tetracycline activity (Burden, 1986). Class N, while genetically uncharacterized, also shows a non efflux mechanism and is probably in this family as well (Burden, 1986). The Class M determinant is widely disseminated among very different bacteria of both aerobic and anaerobic genera, and having different ecological niches and cell membrane structures (Levy, 1988; Roberts, 1989). Originally described in Streptococcus agalactiae, this determinant has been identified in other Gram-positive as well as Gram-negative genera, including Gardnerella and Neisseria spp. It is also the only determinant to date which mediates tetracycline resistance in Mycoplasma and Ureaplasma (Roberts, 1989). Relevant to this spread, Class M is found on transposon Tn916 in streptococci and in Clostridium difficile as well (Hächler, Kayser & Berger-Bächli, 1987). Its presence in multiple chromosomal locations in other organisms strongly suggests that it is also on a transposable element in other genera, e.g. Mycoplasma (Roberts et al., 1985).

Of recent interest is the unexpected finding of two resistance mechanisms associated with a cryptic tetracycline resistance determinant found in Bacteroides fragilis strains expressing resistance to clindamycin. When the clindamycin resistance determinant was cloned on a DNA fragment in E. coli, tetracycline resistance was now expressed but only aerobically (Guiney, Hasegawan & Davis, 1984). Recent work has demonstrated that this resistance involves two separate mechanisms: active efflux (Park et al., 1987) and chemical inactivation of the drug (Park & Levy, 1988; Speer & Salyers, 1988).

There are other tetracycline resistance determinants for which a mechanism and a defined gene product have not yet been identified, e.g. the Class P determinant, so far identified only in C. perfringens (Abraham, Berryman & Rood, 1988); certain unclassified determinants in Pseudomonas spp., (Levy, 1984); and other resistance determinants in fish pathogens (Aoki, Satoh & Kitao, 1987). We have found that the determinant in the chromosome of Proteus mirabilis specifies a tetracycline-inducible active efflux system, but does not hybridize to any of the DNA probes for Classes A–E (unpublished observations).

The spread of tetracycline resistance has provided insight into the diversity of natural gene exchange that occurs in our environment.

Even rare transfers may be enriched and propagated by natural selection, notably by use of the antibiotic. Moreover, once selected, bacteria bearing these determinants are lost very slowly (Levy, 1986). As more and more determinants are being discovered, it is clear that they have not arisen recently in response to tetracycline usage, but have apparently evolved over millennia in response to environmental pressures. While it is possible that these determinants have served bacteria in competition with organisms producing tetracycline-like antibiotics, the finding of cryptic tetracycline efflux systems in the chromosome of E. coli (George & Levy, 1983) and a cryptic gene in Bacil. subtilis which expresses tetracycline resistance only when in multicopy (Ives & Bott, 1989), suggest that these determinants, while able to handle tetracycline, may be present for other purposes in the bacterial cell. Identification of the role of these determinants in natural microbial physiology and ecology might help suggest steps towards controlling their persistence and spread. Other efforts directed at identifying the site for tetracycline interaction with the resistance proteins would help towards designing new tetracyclines not subject to these common resistance mechanisms.

References


Antrimicrobial Agents and Chemotherapy 31, 1739-45. 
Antimicrobial Agents and Chemotherapy 32, 1797-1800. 
Journal of General Microbiology 134, 585-98. 
Antimicrobial Agents and Chemotherapy, 28, 141-145. 
Molecular & General Genetics 215, 76-80. 
Nucleic Acid Research 12, 7693-703. 
Nucleic Acid Research 11, 6089-105.