Tetracycline resistance determinants

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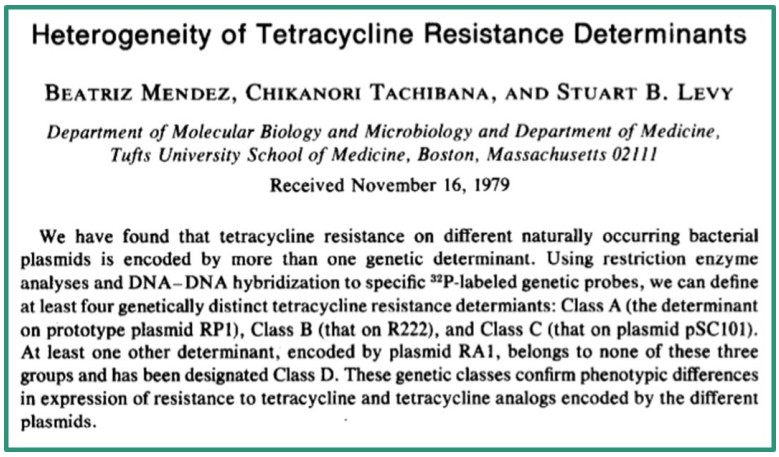
Prof. Levy had a great interest in tetracycline resistance genes in Gram negative bacteria, the various tetracyclines developed, and the history of the field. He has published >75 papers that discuss the active efflux *tet* genes and chromosomal *mar* gene, various tetracycline analogs, as well as, the history of the tetracyclines. One of his first papers in the field was published in *Nature* in 1970 where he studied the segregation of transferable plasmids in *E. coli* minicells. This was 39 years after the first tetracycline compound Aureomycin™ was discovered in the early 1940s by Lederle Laboratories Division of American Cyanamid. This first tetracycline had a wide range of activity. It was a broad-spectrum antibiotic which had activity against Gram positive and Gram negative bacteria and was the first antibiotic to be given this label. Other pharmaceutical companies discovered other tetracycline compounds. Tetracycline was first introduced for clinical therapy in 1948. These antibiotics inhibit protein synthesis by binding to the 30S ribosomal subunit. There are now other broad spectrum antibiotics in different classes of antibiotics.



Heterogeneity of Tetracycline Resistance Determinants

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We have found that tetracycline resistance on different naturally occurring bacterial plasmids is encoded by more than one genetic determinant. Using restriction enzyme analyses and DNA–DNA hybridization to specific ^{32}P-labeled genetic probes, we can define at least four genetically distinct tetracycline resistance determiants: Class A (the determinant on prototype plasmid RP1), Class B (that on R222), and Class C (that on plasmid pSC101). At least one other determinant, encoded by plasmid RA1, belongs to none of these three groups and has been designated Class D. These genetic classes confirm phenotypic differences in expression of resistance to tetracycline and tetracycline analogs encoded by the different plasmids.

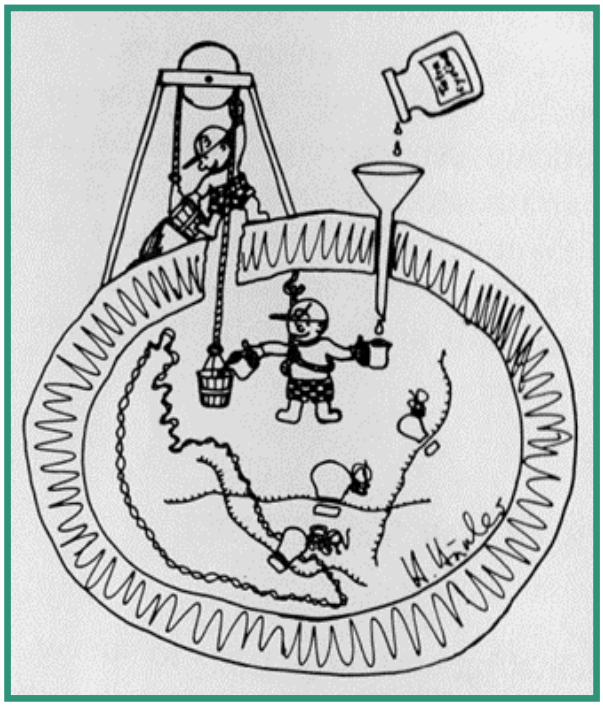
I have chosen two of these tetracycline papers of Prof. Levy to provide a mini-review because they have had a great impact on the tetracycline resistance gene nomenclature as well as other antibiotic resistant nomenclatures as having direct impact on my research career.

Prof. Levy's 1980 paper describes the identification of four genetically distinct tetracycline resistance determinants which were labeled Class A, B, C & D. These four genes were confirmed to have phenotypic differences in expression of resistance to tetracycline, minocycline and chelocardin and were encoded by different plasmids from members of the Enterobacteriaceae and Pseudomonadaceae. These were the first four characterised *tet* genes which conferred resistance by an active efflux mechanism which decreased the accumulation of tetracycline in the host bacterial cell. Previously in Enterobacteriaceae, *Staphylococcus* and some anaerobic species were shown that they were inducibly resistant to higher levels of tetracycline if the hosts had previously been exposed to subinhibitory concentrations of the drug. An inducible negatively regulated protein was identified in the inner membrane of the bacteria carrying one of these genes.

The initial discovery that there are different types of tetracycline resistance genes was a major breakthrough in the field of antibiotic resistance. The four genes were shown to be unrelated using DNA-DNA hybridisation, which was the state of the art for the day. The paper then went on to show the distribution of these different genes using ^{32}P-labeled fragments to determine carriage of the different *tet* genes against 25 different strains representing 12 different species carrying plasmids from different incompatibility groups and different resistant patterns using filter hybridisation. The four genes described in the 1980 paper are the standard for the active efflux *tet* class of genes and are exclusively found in Gram negative genera.

Prof. Levy demonstrated in the 1980 paper that a *tet* gene could be associated with a transposon, in this case the highly studied Tn*10*. The *tet*(B) gene encoded conferred resistance to minocycline while the other three efflux genes did not.

Today we know that these efflux genes are regulated by a specific repressor gene which is upstream of the structural gene and is read in the opposite direction from the structural gene. Today there are 33 genetically distinct *tet* efflux genes in this class of *tet* resistance genes — many of great clinical significance[3.] Today we know that these *tet* genes are α-helices that are divided into two halves, α and β, by a large putative cytoplasmic loop designated the interdomain region. The α and β domains of the protein (N-terminal and C-terminal halves, respectively) have presumably evolved from a duplication of a single domain. A number of the *tet* genes including the first four described in the 1980 papers have 45–75% identity. Hybrid interclass Tet protein constructions, complementation studies and second-site suppressor studies showed that interactions between both domains are required for function.



The molecular methods described in Levy's 1980 paper went on to become the standard method for surveillance of the distribution of various tetracycline resistance genes. Similar methods were used to identify heterogeneity in *tet* resistance genes in *Streptococcus* spp. The methods changed with the introduction of polymerase-chain reaction (PCR) assays in the 1990s for the detection of different tetracycline resistance genes.

In the second paper, a short publication, Prof. Levy worked with others in the field to define a nomenclature system for *tet* genes which still works today some 30 year later. From this work the tetracycline resistance gene nomenclature center was borne[3.] Prof. Levy's laboratory developed a form for authors to fill out so that they could request new names for newly identified genes that were <80% related by amino acid identity with other *tet* genes previously described and given names. The aim of this paper and the centre was to make sure that highly related *tet* genes were given the same name. At this time, <80% identity was the best discrimination that could be done. Names were then provided and a website was developed to provide the information free to all who were interested. At the same time, I modeled the nomenclature for macrolide -lincosamide-streptogramin genes after the *tet* system and provided a website for these genes as well. Today there is a total of 60 different *tet* genes with other mechanisms including ribosomal protection [n=13], enzymatic inactivation of the antibiotic [n=13] and one with unknown mechanism of action.

References

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3. Marilyn C Roberts PhD: Nomenclature for Tetracycline Genes and Nomenclature Center for MLS Genes. https://faculty.washington.edu/marilynr/