Aspersing aspirin: Salicylate-inducible antibiotic resistance

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One outcome that results from the exposure of microorganisms to antibiotics in their environment is the selection of resistant mutants. The origin of such mutants can be traced back ultimately to random errors in DNA replication. Another possible outcome is the emergence of variants which are genetically wild type, but phenotypically adapted to the adverse environment. In such cases the resistance is not selected but is induced. The distinction between selection and induction may not always be clear-cut. The possibility that the wild type may be programmed to 'induce' adaptive mutations under certain stresses is again attracting serious scientific attention[1]. Conversely, one can imagine the selection of mutants that are more efficient at inducing the tolerance phenotype. The problem then is to understand how different inducible resistance mechanisms get turned on. One instance in which this problem has resulted in a tale with surprising twists is the induction by salicylate of antibiotic resistance in gram-negative bacteria.

The first report of salicylate-inducible antibiotic resistance was that in 1985 by Judah L. Rosner, who showed that *Escherichia coli* K-12 cells become significantly tolerant to chloram-phenicol, tetracycline, ampicillin and nalidixic acid in the presence of millimolar concentration of chemorepellents such as salicylate, acetate, acetylsalicylate (aspirin), benzoate, dimethyl sulfoxide and 1-methyl 2-pyrrolidinone[2]. The cells reverted completely when returned to chemorepellent-free medium. It is striking that these antibiotics have diverse modes of action and that their structures are not related to that of the tolerance inducing chemorepellents.

In 1987 Sawai *et al.*[3], reported that growth in the presence of salicylate drastically reduced the OmpF porin content of the outer membrane of *E. coli*, *Klebsiella pneumoniae* and *Serratia marcescens*. Since the OmpF porins are largely responsible for permeation through the outer membrane of low-molecular-mass (<600 Da) hydrophilic molecules (including the antibiotics mentioned above), it was reasonable to infer that the resistance phenotype induced by salicylate was a consequence of reduced uptake of the antibiotics due to the loss of OmpF porins from the outer membrane. Thus the original question of how salicylate induces antibiotic resistance became sharpened into the more focussed one of how salicylate reduces OmpF porin expression.

Using various *ompF-lacZ* fusion strains, Rosner *et al.*[4] addressed this latter question by examining the effects of salicylate on the transcription and translation of the *ompF* gene. The *lacZ* gene codes for the enzyme β-galactosidase whose activity can be easily assayed. If promoterless *lacZ* sequences are fused to the *ompF* promoter and this fusion is introduced into a strain that is deleted for the normal *lacZ* locus, then β-galactosidase activity in this strain provides a measure of the *ompF* promoter's activity. Such *ompF-lacZ* fusions are called transcriptional fusions. If the promoterless *lacZ* sequence also lacks the ribosome binding site and is fused downstream of the *ompF* translation start site in the correct reading frame to yield an *ompF-lacZ* fusion protein with β-galactosidase activity, then the *ompF-lacZ* fusion is called a translational fusion and the β-galactosidase expression is a measure of both transcriptional and translational activity of *ompF*. Rosner *et al.*, found that salicylate had no effect on β-galactosidase activity from the transcriptional fusions, but two translational fusions showed 12- to 15-fold decreases in β-galactosidase activity in the presence of salicylate. From these results they could conclude that salicylate reduced *ompF* expression via a post-transcriptional effect.

OmpF porin expression was previously shown to be subject to post-transcriptional regulation by the product of the *micF* locus[5]. The *micF* locus does not contain translational open reading frames but codes for a micRNA. micRNA is the acronym carefully chosen by Mizuno, Chou and Inouye for messenger RNA interfering complementary RNA (i.e., RNA molecules with sequence complementarity to transcripts of particular genes and therefore able to bind as an antisense RNA and prevent the translation of bound transcripts). Rosner *et al.*[4], therefore constructed *micF-lacZ* fusions, and used them to show that salicylate induced *micF* transcription. Since *micF* transcripts inhibit the translation of *ompF* transcripts one

could now account for salicylate-induced decrease in OmpF porins. Thus, the original question of how salicylate induced antibiotic resistance appeared to be solved. Actually, the old question was replaced by a new one; how does salicylate increase *micF* transcription?

'Solutions' are seldom clean. If indeed salicylate-induced reduction of OmpF was mediated only via *micF*, salicylate should have no effect on OmpF levels in a *micF*-deleted strain. However, Rosner *et al.* found that even in the absence of *micF*, salicylate could reduce the amount of OmpF in the outer membrane although greater concentrations of salicylate were required for this effect. Thus the absence of *micF* diminished the inhibitory effects of salicylate but did not abolish it completely. This implicates an additional non-*micF* anti-*ompF* translational activity in the salicylate response. A newly identified *micF* RNA-binding protein which can also bind to *ompF* mRNA may be a good candidate[6].

As if this was not complex enough, Rosner and coworkers found that salicylate *increased* the sensitivity of cells to aminoglycoside antibiotics (e.g. kanamycin, neomycin, tobramycin, kasugamycin)[7]. Here again salicylate appears to have two modes of action — one due to its behavior as a weak acid that raises membrane potential at low pH and thereby facilitating uptake of aminoglycosides, and the other a pH-independent action that appears to be related to its salicyl structure. In this latter mode of action the salicyl structure (one that is shared by salicylate and salicyl alcohol) may chelate divalent cations that are antagonistic to aminoglycoside activity or it may have some other regulatory effect on the cell.

Recently, Burns and Clark[8] have shown that salicylate decreases *opcS* porin synthesis in *Pseudomonas cepacia* and thereby induces resistance to the antibiotics chloramphenicol, trimethoprim and ciprofloxacin. *Pseudomonas cepacia* is a ubiquitous bacterium which has been responsible for outbreak of nosocomial infections and for severe pulmonary infection in individuals with cystic fibrosis. Since salicylates are the most widely used antipyretic analgesic drugs (and therefore most likely to be administered at the onset of a fever) the problem of salicylate-inducible antibiotic resistance is, unfortunately, not merely academic.

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