Erythromycin dependence and RNA polymerase.

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In Escherichia coli (and other bacteria), antibiotic resistance can be acquired by chromosomal mutations, often in genes coding for ribosomal proteins. Mutations can also generate organisms that now require an antibiotic for growth. For example, streptomycin resistance or dependence both result from single amino acid changes in ribosomal protein S12 [1]. However, other examples of antibiotic dependence are much less studied.

Two strains have been described that require erythromycin for growth. Both throw off erythromycin-independent 'revertants' at similar frequencies (about 1 per 10^6 organisms). Revertants of strain AM [2] often have a ribosomal protein missing and so have been used to help locate and explore the function of that protein on non-mutant ribosomes [e.g. 3]. Similarly, some revertants of strain 103 [4] have altered or missing ribosomal proteins and are defective in ribosome assembly and subunit association. In neither strain has the nature of the dependence mutation been established.

Strain AM has an unusual phenotype [5]. Without antibiotic, organisms grew for only 1-2 generations in liquid medium. Erythromycin supported continued exponential growth; so did other antibiotics but only within a narrow concentration range that had little effect on the growth of the parent of strain AM. The antibiotics included inhibitors of translation (chloramphenicol, spectinomycin, tetracycline, kanamycin) and transcription (rifampicin). The effect of spectinomycin, for example, was mediated through ribosomes because a spectinomycin-resistant derivative of strain AM with an altered ribosomal protein S5 required more spectinomycin than strain AM to support the dependency. However, RNA polymerase was similarly implicated because rifampicin-resistant derivatives of strain AM required higher concentrations of rifampicin than strain AM in order to grow. Rather erratic growth of strain AM was also possible without antibiotics in some media of high ionic strength.

Kanamycin did not support growth of strain AM. Transposition with bacteriophage lambda467 (carrying the kanamycin-resistant transposon Tn5) was therefore used to isolate a pool of kanamycin-resistant wild-type organisms. Then, clones were isolated that, in transductions with bacteriophage P1, transferred kanamycin resistance and erythromycin independence simultaneously into strain AM. Conjugation located kanamycin resistance in these clones at about 85-90 min on the bacterial chromosome. Transductions with P1 showed that the dependence mutation in strain AM was between pheS (89.5 min) and purD (90.4 min) and was extremely closely (98%) linked to, and clockwise of, a rifampicin-resistance mutation in the β-subunit of RNA polymerase (rpoB). Rifampicin-resistance mutations cluster about 1.5 kb from the proximal end of the 4kb rpoB gene, so that the dependence mutation is likely to be in rpoB (or, less likely, in the downstream rpoC).

We speculate that the dependence mutation in strain AM affects polymerase function in such a way as to alter the spacing between the enzyme and the leading ribosome in coupled transcription-translation. 'Correct' spacing can be re-established by an antibiotic that restores polymerase function (rifampicin) or slows down ribosome movement (e.g. erythromycin, spectinomycin) or by mutations ('reversion') that reduce ribosome function.

These suggestions are being tested. Even if correct, they are not a universal explanation of erythromycin-dependence. Strain 103 has a different and less flexible phenotype than strain AM. In particular, rifampicin did not support growth and only chloramphenicol, tetracycline and spectinomycin would substitute for erythromycin, the latter two with difficulty. Transpositions with Tn5 positioned a kanamycin-resistance marker close to the independence allele in a wild-type strain. Conjugation experiments placed the kanamycin resistance at about 40 min. The dependence mutation could then be linked by P1 transduction to pheS (37.2 min), purD (37.3 min) and pheS (37.4 min). Although no more precise position has yet been established, both the phenotype and genotype of strain 103 are different from that of strain AM.