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Leading articles

Genetics of methicillin resistance in *Staphylococcus aureus*

Methicillin resistant (Mc^r) staphylococci emerged in the early 1960s, soon after the first β-lactamase-resistant penicillins were used clinically. The observation of similar phage types among Mc^r strains and the association of methicillin resistance with specific resistance patterns led to the hypothesis that Mc^r staphylococci spread initially from a single clone of Mc^r *Staphylococcus aureus*. Later the phage type changed and the methicillin resistance was found concomitantly with additional or different resistance determinants. This diversification of Mc^r staphylococci might be due to the appearance of new Mc^r strains or to lysogeny, conjugation, and exchange of plasmids and transposable elements carrying resistance and virulence determinants between strains. Nowadays, methicillin resistance in *S. aureus* is often found to be associated with multiple resistance.

The methicillin resistance determinant (*mec*) confers an intrinsic resistance to the action of β-lactam antibiotics. For a long period of time the genetic location of *mec* remained controversial. On the one hand *mec* showed properties pointing to a plasmid origin; on the other hand no physical linkage with any specific plasmid could be demonstrated. Kuhl, Pattee & Baldwin (1978) finally showed that *mec* mapped on the chromosome. In cotransformation studies *mec* was linked to the chromosomal marker *pur*A. Results of cotransductional studies by Stewart & Rosenblum (1980) suggested that *mec* was located on additional DNA present in Mc^r strains only and absent in isogenic sensitive strains. Physical demonstration of this additional DNA was presented by Beck, Berger-Bächi & Kayser (1986). There was evidence that *mec* might be transposable, because the ability of *mec* to integrate into the chromosome was independent of the host recombination system. Furthermore, Trees & Iandolo (1988) captured a transposon (Tn*4291*) on a penicillinase plasmid that carried methicillin resistance genes. These findings would explain the intriguing behaviour of *mec*, evading precise localization by genetic means during the first years after its discovery.

Part of the additional DNA coding for *mec* has been cloned by different groups (Beck *et al.*, 1986; Matsuhashi *et al.*, 1986; Matthews, Reed & Stewart, 1987). A specific region of this additional DNA was found to be conserved in all Mc^r strains of *S. aureus* and coagulase-negative staphylococci collected during the past 25 years (Berger-Bächi, B. Beck, W. D., Pitzko, D. & Kayser, F. H., unpublished results). This DNA region codes for a novel penicillin-binding protein (PBP) with low affinity for penicillin. This novel low-affinity PBP was identical with PBP2′ (or PBP2a) that had previously been found by different groups to be added to the usual set of PBPs in Mc^r *S. aureus* (Hartman & Tomasz, 1984; Utsui & Yokota, 1985). PBP2′ is the only PBP functioning in cell wall synthesis at β-lactam antibiotic concentrations high enough to inhibit growth of sensitive strains. PBP2′ plays an essential role in methicillin resistance of staphylococci since introduction of a DNA fragment containing the structural gene for PBP2′ into a sensitive staphylococcal host resulted in expression of methicillin resistance (Inglis, Matthews & Stewart, 1988; Tesch *et al.*, 1988). The gene coding for this protein was sequenced (Song *et al.*, 1987), and it was thought to have evolved from two different genes, an inducible penicillinase gene and a PBP gene of unknown origin. The question whether the structural gene for PBP2′ synthesis is present on the putative *mec*-transposon Tn*4291* mentioned above, however, is still open.

Besides PBP2′, additional factors are involved in the expression and regulation of *mec*. PBP2′ synthesis is constitutive but becomes inducible in the presence of certain penicillinase plasmids. Furthermore, a factor on the chromosomal segment number 18, genetically very distant from *mec*, was found to be essential for Mc^r expression, without affecting PBP2′ synthesis. Inactivation of this factor by Tn*551* insertion resulted in loss of *mec* expression (Berger-Bächi, 1983; Berger-Bächi, Strässle & Kayser, 1986). Preliminary results (Kornblum *et al.*, 1986; Berger-Bächi,

B., Barberis-Maino, L., Strässle, A. & Kayser F. H., unpublished results) suggest at least three different chromosomal sites to be involved in the control of *mec*.

In most clinical isolates of Mc^r *S. aureus* the majority of the population shows a low basal resistance to methicillin, but a few cells, 10^{-4} to 10^{-6} of the population, are resistant to higher levels of methicillin. At present, no genetic model is able to explain this intriguing heterogeneity. Matthews & Stewart (1988) described amplification of sections of chromosomal DNA of Mc^r *S. aureus* after selection for increased resistance to methicillin. However, the amplified regions were lost upon prolonged storage of the variants, without concomitant loss of methicillin resistance.

One insertion-sequence-like element (IS*431*) was found to be associated with the *mec*-specific DNA (Barberis -Maino *et al.*, 1987). However, since this IS-like element was surrounded by the additional *mec*-specific DNA on the chromosome of the Mc^r staphylococci, it is probably not responsible for the integration of the putative *mec* transposon (Tn*4291*) into the chromosome. The same IS-like sequence was also found elsewhere on plasmids, associated with, or in proximity to, other resistance determinants, and sometimes on the chromosome. In a multiresistant clinical isolate of Mc^r *S. aureus*, Gillespie *et al.* (1987) found four direct repeats of an indistinguishable IS-sequence, there called IS*257*, which was associated with the methicillin resistance determinant. They were flanking a mercury resistance determinant as well as a tetracycline plasmid that had integrated into the chromosome adjacent to *mec*. A tobramycin resistance determinant was also found to be tightly linked with *mec* in certain Mc^r *S. aureus* strains (Ubukata *et al.*, 1985). These observations suggest that the *mec*-specific DNA may function as a trap for resistance determinants by providing them with a recombination site, maybe in the form of IS*431*, for their integration into the chromosome.

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The enigma of streptomycin transport

Professor Davis recently reviewed in this Journal (Davis, 1988) his stimulating hypothesis concerning the transport of streptomycin (and dihydrostreptomycin which is believed to behave biochemically in an identical manner to streptomycin) into bacterial cells (Davis, Chen & Tai, 1986; Davis, 1987). In this hypothesis, Davis proposes that mis-translated proteins accumulate in the cytoplasmic membrane and form transmembrane aqueous channels that allow the positively charged antibiotic molecules to diffuse from the exogenous compartment into the cytoplasm. In his leading article, Davis (1988) also pointed out that one of the challenges still facing this hypothesis was that of explaining the kinetically irreversible nature of dihydrostreptomycin transport (Nichols & Young, 1985) which implies a more specific transport process than that outlined above (Nichols, 1987). I propose to elaborate briefly in this article why the irreversibility of transport should be seen as being inconsistent with the hypothesis of water-filled channels. For further detailed information on bacterial aminoglycoside transport, the reader is also referred to the comprehensive review by Taber *et al.* (1987).

First of all, it seems clear that the primary inhibition of bacterial growth caused by streptomycin is due to the antibiotic causing ribosomes to participate in cyclic formation and dissociation of non-functional 'initiation complexes' (Tai & Davis, 1985; Davis, 1987); which in antibiotic-free cells would go on to carry out full protein synthesis. It also seems to be clear that the bactericidal nature of streptomycin action is due to its not being lost from the cell on removal of external antibiotic (Davis, 1987; Nichols, 1987; Taber *et al.*, 1987): that is, the uptake process is, as far as can be measured, kinetically irreversible (Nichols & Young, 1985). Moreover, because of the intimate link between dihydrostreptomycin transport and the action of the antibiotic on the ribosome (e.g. see Hurwitz, Braun & Rosano, 1981), it seems reasonable to suggest that the other action of dihydrostreptomycin (that of causing mis-reading during polypeptide elongation) occurs at sites of membrane protein synthesis soon after exposure of cells to the antibiotic, and that this mis-reading activates the process of further transport of antibiotic into the cytoplasm (Davis, 1987, 1988). However it is here that the 'channel' hypothesis runs into difficulties (Nichols, 1987).

It is perhaps useful to summarize the key features of the 'channel' hypothesis. Davis (1987, 1988) ascribes particular importance to some observations made more than 25 years ago (Dubin, Hancock & Davis, 1963). One of the earliest events that occurs coincidentally with, or shortly after, initiation of rapid energy-dependent uptake of streptomycin by *Escherichia coli* is the loss of K^+ and nucleotides from the cell cytoplasm into the external medium (Dubin *et al.*, 1963). Davis (1987, 1988) interprets these observations as being the result of the cytoplasmic membrane becoming generally leaky; that is, possessing channels through which the K^+ and nucleotides diffuse. This is proposed to be due to one of the established actions of streptomycin, that of causing mis-translation during protein synthesis, occurring at ribosomes synthesizing membrane or exported proteins. Specifically, Davis *et al.* (1986) have suggested that these mis-translated proteins accumulate in the cytoplasmic membrane and cause generalized leakiness there. This proposed generalized membrane leakiness is also taken to explain the onset of the rapid, respiration-dependent transport of streptomycin into the cytoplasm. Positively-charged molecules of streptomycin are proposed to diffuse inward, in response to the electric potential gradient (Damper & Epstein, 1981), through the transmembrane aqueous channels responsible for the generalized leakiness (Davis, 1987; 1988).

The problem is that one would not expect channels that link the two aqueous compart-