Response to Hancock

Hancock (2003) makes two important points: that widespread resistance to host peptides has not evolved over evolutionary time, and that levels of resistance to industrially produced peptides such as nisin and polymyxin remain low. These are indeed grounds for optimism. At the same time, although it was no part of the intention of the original article to discourage research into a promising approach to the control of infectious disease, a certain degree of caution is in order.

Levels of resistance to naturally occurring RAMPs (ribosomally synthesized antimicrobial peptides) indeed remain very low, so they retain their efficacy as antimicrobial systems. This might be because most bacteria are, for whatever reason, constitutionally unable to express effective defences against them, with the result that the same systems continue to be effective for millennia. On the other hand, the roughly $5 \times 10^4$ species of vertebrates may collectively produce as many as $10^6$ different RAMPs, constituting an extremely diverse environment in which sustained selection for specialized resistance may not often occur. It is, moreover, a moving target. Recent work (Vanhoey et al., 2003) has shown that the great diversity of RAMPs found in hylid frogs are modified from precursors that are evolutionarily conserved, except for a hypervariable region that forms the mature peptide. The authors speculate that this may be produced through a defective and thus mutagenic polymerase. Rather than evolutionary stasis maintained by physiological or genetic constraints, this suggests a highly dynamic system in which virulence and resistance continually shift as the result of a co-evolutionary arms race between the frogs and their bacterial pathogens. It also implies, however, that while resistance to RAMPs may evolve more readily than expected it is also rather highly specific, with little cross-resistance. The comparative biology of RAMPs deserves to be explored more extensively.

Nisin is a peptide produced by *Lactococcus* that has been used for 50 years to preserve milk products and canned vegetables at concentrations of about 100 mg kg$^{-1}$. Resistance is well-documented but the frequency of resistance nevertheless remains low (Montville et al., 2001; Davidson & Harrison, 2002). Again, it may be that resistance is intrinsically unlikely to arise. There is another possibility, however. If resistance were to arise in a can of soup, for example, the food would be spoiled and the can discarded. The spoiled can is an evolutionary dead end, because there is no easy route by which a mutant strain infecting one can could be transmitted to other cans and thus spread widely through the population. It may be, therefore, not that resistance is unlikely to arise, but rather that it is unlikely to spread. For other industrial uses, this may not be the case. Several authors have recently reported success in expressing transgenes encoding vertebrate RAMPs in plants (DeGray et al., 2001; Ponti et al., 2003), and these may in future be used to confer pathogen resistance on genetically modified crop plants. In these circumstances, a resistant lineage could spread rapidly over wide areas, forming
large populations capable of releasing dispersive spores into the general environment. The evolution of resistance, then, depends not so much on the molecular biology of an organism but rather on the evolutionary biology of its interaction with the environment.

Whether resistance to RAMPs can be expected to evolve and the extent to which cross-resistance is likely to be a correlated response are empirical issues that are unlikely to be resolved decisively by argument but must instead be investigated through selection experiments. Those that have so far been published are less than completely satisfactory. The study by Steinberg et al. (1997) cited by Hancock (2003) took methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa through 11–18 transfers at 50 % of the MIC, and obtained a response to norfloxacin and gentamicin but not to vancomycin or pig protegrin-1. The experiment is too brief to be completely convincing, and certain technical details, such as the number of selection lines used, were not reported. Systematic selection experiments will begin shortly at McGill University, and an account will be published subsequently in the technical literature – whatever the outcome.

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