PROBING THE EVOLUTIONARY ORIGINS OF BACTERIAL TRANSLATION INITIATION USING EXPERIMENTAL EVOLUTION. A. M. Poole[1,2,3], J. A. Heinemann[1] and R. J. Catchpole[1,2], [1]School of Biological Sciences, [2]Biomolecular Interaction Centre, [3]Allan Wilson Centre for Molecular Ecology & Evolution, University of Canterbury, Christchurch, New Zealand (anthony.poole@canterbury.ac.nz).

The protein synthesis machinery evolved extremely early in the history of life. Despite this antiquity, there are fundamental differences between bacteria, archaea and eukaryotes in terms of how translation is initiated. These differences may be associated with beneficial, lineage-specific selection on the populations that evolved into the three domains. However, some features of translation initiation are puzzling, and difficult to associate with any clear benefit. We believe this is the case for the use of formylmethioinine in bacterial translation initiation. Briefly, bacterial translation initiation utilises formylmethionine, which is generated through formylation of charged initiator tRNA. Curiously, the formyl group is removed during protein synthesis, and this cycle of addition and removal is not a feature of either archaeal or eukaryotic translation initiation.

We subjected a double knockout strain of *E. coli*, which lacks formylmethyltransferase and peptide deformylase genes (required respectively for formyl group addition and removal), to a long-term evolution experiment to establish whether *E.coli* lacking these genes can evolve to undertake normal rates of translation. Following approximately 2000 generations of culturing, we derived lines capable of wild-type growth. We then performed whole genome sequencing of 11 wild-type control lines and 11 evolved double knockout lines. We identified parallel mutations in the translation machinery of the double knockout lines, which enable bacteria to be cured of formyl addition and removal. Given the oddly futile cycle of addition and removal, we next asked whether, when plasmid-borne, the formylmethyltransferase and peptide deformylase enzymes can act as an addiction system capable of eliciting post-segregational killing. In post segregational killing, a plasmid, upon gaining entry into a cell, expresses a toxin and an antitoxin. Cells that subsequently lose the plasmid undergo cell death as the toxin outlasts the antitoxin. Consequently, this provides an effective means of spread and persistence through a bacterial population. We find that, in our evolved strains, reintroduction of the formylation and deformylation enzymes results in a post-segregational killing phenotype.

Our results suggest an evolutionary origin for this modified form of translation initiation via post-segregational killing. Finally, we introduced these same genes into yeast, and show that addition of these genes to the nucleus does not elicit the same addictive phenotype. We will explain how this helps account for the observation that this process is limited to eukaryote mitochondria and plastids, despite ongoing endosymbiont gene transfer to the nucleus.