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SYNTHETIC BIOLOGY

Genetic kill switches — a matter of life or death

A study in *Nature Chemical Biology* reports the development of two new strategies to control genetically engineered *Escherichia coli* on the basis of modular, reprogrammable genetic circuits.

The applications of genetically engineered bacteria are diverse and ever-expanding, but the possibility that these organisms escape confinement poses an environmental threat. Therefore, several approaches have been developed to make bacterial survival dependent on the presence of non-native or non-natural components in the culture medium. However, some of these approaches could fail if the metabolites required for growth are unexpectedly present in uncontrolled environments, and those in which the bacterial genome needs to be modified on a large scale might be impractical and cumbersome.

The first strategy, dubbed 'Deadman', relies on a circuit in which the LacI and TetR transcription factors are reciprocally repressive, but in which the expression of TetR is favoured owing to modifications in the strength of the ribosomal binding sites of the two transcription factors. Inhibition of TetR expression by anhydrotetracycline (ATc), a compound that is not normally found in nature, is necessary for expression of LacI. LacI directly inhibits expression of a lethal toxin and/or indirectly prevents inhibition of the expression of an essential gene; these effects, either alone or combined in a single circuit, keep the cells alive. Removal of ATc from the environment activates the expression of TetR, which leads to cell death. A 'fail-safe' mechanism was also added to the system, whereby production of the toxin and cell death are independently activated by isopropyl β-D-1-thiogalactopyranoside (IPTG).

The second strategy, named 'Passcode', is based on the construction of fusions of environmental sensing modules of specific transcription factors and DNA-recognition modules of different transcription factors. Hybrid transcription factors with the same DNA-recognition module but with different environmental sensing modules can therefore be built. The researchers used three different hybrid transcription factors to build a circuit in which the concomitant presence of two distinct environmental cues and the absence of another environmental cue are simultaneously required for preventing expression of a toxin and, thus, for cell survival.

Cultivation of the cells engineered with the 'Deadman' or the 'Passcode' systems for 4 days showed that the killing efficiency of the systems decreased with time. However, increasing the stability of the host genome by deleting insertion-sequence elements, which cause inactivating mutations in the toxin-encoding genes, preserved the functionality of the killing systems for a substantially longer period.

Owing to their intrinsic potential for customization, 'Deadman' and 'Passcode' are promising new biocontainment tools. In addition, the researchers suggest that the synthetic gene circuit 'Passcode' might be particularly useful to protect intellectual property as it relates to bacterial strains, as the appropriate 'passcode' molecules are needed to ensure bacterial survival.

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The author declares no competing interests.

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