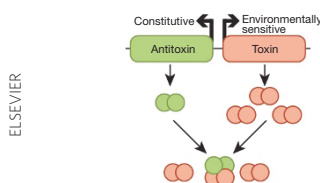


SYNTHETIC BIOLOGY

License to kill

Mol. Cell **68**, 686–697.e3 (2017)



Bacterial kill switches involve induction of lethal genes unless certain environmental conditions are met, and can be used to contain genetically engineered microorganisms and prevent the spread of their engineered genes. Stirling *et al.* have now developed two new kill switches for use in *Escherichia coli*, termed ‘essentializer’ and ‘cryodeath’, that utilize a toxin–antitoxin system in which toxin CcdB is expressed if certain environmental conditions are not met, while antitoxin CcdA is constitutively expressed to counteract any leaky CcdB expression when those conditions are met. The essentializer system maintains the presence of a previously developed ‘memory element’ that records and maintains a signal output through the use of two transcription factors. If the memory element is lost and neither transcription factor is present, the essentializer system induces production of CcdB and the cell dies. Meanwhile, the cryodeath system represses CcdB expression at 37 °C, but induces it at lower temperatures. Application of cryodeath in the mammalian gut ensures that the engineered bacteria are no longer viable once excreted into the environment. By extensively

screening libraries of genetic elements that make up these kill switches, the authors have developed systems that are particularly stable to evolutionary pressures and that can be maintained for many generations without continuous input of exogenous survival factors.

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as can long-lived proteins and proteins in primary cells, for which RNAi experiments are challenging. Trim-Away will open up new avenues for research because it is an easily applicable tool for studying protein function that can be used for most intracellular proteins.

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CELL BIOLOGY

Eaten up from the inside

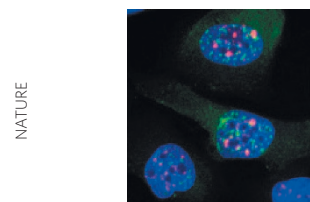
Cell **171**, 1692–1706 (2017)

The antibody receptor and ubiquitin ligase TRIM21 recognizes antibody-bound pathogens and triggers their proteasomal degradation. Using TRIM21, Clift *et al.* developed a new post-translational protein knockdown approach called Trim-Away in which an antibody specific for the protein of interest is introduced into mammalian cells either through microinjection or electroporation, leading to TRIM21-mediated specific and rapid degradation of the target protein within minutes. In some cell lines, endogenous TRIM21 levels are sufficient to achieve degradation; alternatively, TRIM21 can be heterologously expressed in cells, or purified TRIM21 can be inserted together with the antibody by electroporation. Both cytosolic and membrane-anchored proteins can be targeted by Trim-Away in this manner, and nuclear proteins can also be selectively degraded by using a nanobody fused to the Fc domain of an antibody, which is required for TRIM21 recognition. The authors demonstrate the promise of this method by showing that specific variants of a protein, for example a disease-causing form of huntingtin, can be selectively degraded,

CANCER THERAPY

A path of DSF destruction

Nature **552**, 194–199 (2017)



Disulfiram (DSF) is a drug used for treatment of alcohol dependency through inhibition of liver aldehyde dehydrogenase, but it also shows activity against a broad spectrum of malignancies. The antitumor activity of DSF is enhanced by its chelation of copper. To determine the direct molecular target responsible for the anticancer effect of DSF, Skrott *et al.* first developed an *in vivo* HPLC–MS approach, through which they found that mouse tumors preferentially accumulate a complex between the DSF metabolite DTC and copper (CuET), with high toxicity against targeted cells. Although CuET induces phenotypes similar to those seen with proteasome inhibitors, including accumulation of polyubiquitylated (polyUb) proteins, it did not inhibit 20S proteasome-dependent protein turnover or 26S proteasome activity. Instead, CuET inhibited processing of polyUb proteins by p97, which acts upstream of the proteasome and has protease and segregase activities. Experiments with p97 inhibitors using fluorescence recovery after photobleaching (FRAP) indicated that CuET inhibits the p97 segregase activity that extracts polyUb proteins from cellular structures. More specifically, CuET inhibits the NPL4 component of the segregase, inducing its clustering in the nucleus of treated cells, which is dependent on a putative zinc finger within the protein. These results support a model wherein CuET binding to NPL4 induces its immobilization and disruption of activity, ultimately leading to cell death, and provide a therapeutic option for tumors that are dependent on p97.

MB

Written by Mirella Bucci, Caitlin Deane, Karin Kuehnel & Stéphane Larochelle

RNA SPLICING

Making the cut

Nat. Commun. **8**, 2100 (2017)

Alternative mRNA splicing is a highly regulated process that is often disrupted in cancer, wherein core components of the pre-mRNA splicing machinery may also be mutated. Several families of natural compounds display antitumor activity that is thought to depend on their action on core components of the pre-mRNA splicing machinery. In particular, spliceostatin A (SSA) and its synthetic derivatives meayamycin and the sudemycins were previously identified to target SFB1, which is involved in 3′ splice-site recognition. To gain detailed insights into how specific drugs modulate splicing and cancer cell viability, Vigevani *et al.* first established that sudemycin C1 can affect alternative splicing events of two genes—MCL1 and PDCD10—to different extents, and that the differential effect was imparted by specific sequences surrounding the splice sites. The authors next assessed the effect of the structurally related SSA and two sudemycins on transcriptome-wide splicing by sequencing both steady state and nascent RNA following drug treatment. Although SSA was more potent than the sudemycins for inducing exon skipping, the sudemycins had a stronger effect on the process, and those effects were modulated by sequence context. Though the precise molecular mechanisms behind these drug-specific effects on splicing remain unclear, the results suggest that it may be possible to identify drugs that selectively regulate splicing to be therapeutically beneficial.

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