CRISPR-Cas toxin-antitoxin systems: selfishness as a constructive evolutionary force.

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A recent paper reports a novel RNA-based Cas-dependent toxin-antitoxin system, with the effect of 'addicting' cells to the cassette. Broadly-defined addiction systems could stabilize diverse genomic features, raising the question of the role of selfish elements and intragenomic conflict in the evolution of biological complexity.

Evolution generally lacks foresight: because evolution proceeds by the cumulative expansion of genotypes that succeed in the present generation, features not of immediate utility can be lost from the population, particularly when those features impose short-term costs. Most clearly, loss may affect features that may be directly useful to future generations, from antibiotic resistance to sexual reproduction to metabolic capacities to entire life stages [1]. Less obviously, loss may affect features that could facilitate the future evolution of adaptive novelties [2]. Such features range from initially redundant gene duplicates, to introns necessary for the future evolution of alternative splicing, to cell type diversity that may be coopted in the future evolution of multicellularity [3]. Given this vulnerability to loss for both current functions and the substrate for future innovations, it is of great interest to understand how nonessential elements are stabilized through evolution.

A recent paper in *Science* provides a fascinating case study of both functional retention and functional elaboration [4]. Li and coauthors report a remarkable archaeal CRISPR-Cas cassette that represents a highly atypical toxin-antitoxin system, though differing in many ways from a classical toxin-antitoxin system (Figure 1A). CRISPR-Cas systems are maintained in many organisms despite imparting ongoing costs but only conditional benefits. The authors report a CRISPR-Cas cassette with modifications that putatively ensure its maintenance (Figure 1B). Nestled within standard CRISPR-Cas components are two remarkable mechanistic novelties: (i) CreT, an unprecedented toxic RNA, which functions by sequestering a rare tRNA; and (ii) CreA, a noncanonical Cas-associated RNA, which serves as an antitoxin by repressing CreT expression through imperfect Cas-dependent basepairing to the *creT* promoter. This genetic contraption provides a remarkable mechanism for maintenance of a putatively conditionally costly genetic element, requiring Cas expression in order to avoid toxic CreT expression. This case also exemplifies the evolution of novel functions from pre-existing features, with the DNA/RNA cleavage Cas machinery having secondarily evolved functions in gene regulation. These results add to the diverse set of cases of entangled evolution of mobile genetic elements and host systems [5]. In addition, this case underscores the parasitism-symbiosis spectrum's dependence on relative costs and benefits in a specific environment, echoing endosymbiotic Wolbachia that encode both viral

defense and toxin-antitoxin systems [6] and transposable elements with both putative costs and roles in gene expression[5].

The work of Li et al. should spur exploration of the diversity of toxin-antitoxin systems in maintaining conditionally functional genetic entities. It is also interesting to speculate about the broader evolutionary relevance of addiction-like systems. Like the toxins of toxin-antitoxin systems, costs associated with selfish transposable elements (TEs) promote maintenance of host cellular machinery to ameliorate these costs, with coping mechanisms ranging from DNA methylation to small RNA-based repression of gene expression to suppression of nonallelic recombination. As with the reported novel CRISPR regulatory function, each of these putatively TE-driven cellular machineries has also been coopted for a range of host functions [5]. These include DNA methylation and RNA-based gene repression, which have contributed greatly to the expanded regulatory complexity of eukaryotic genomes, and efficient repression of nonallelic recombination, which may be important for ubiquitous polyploidization in plants [7]. TE proliferation likely also has less obvious implications. For instance, animals' exon-centered splicing system (so-called "exon definition"), thought to be important for proteome expansion through alternative splicing, was likely driven by intronic expansion through the insertion of TEs [8].

The toxin-antitoxin-like systems in these TE-associated cases differ from classic toxin-antitoxin systems, as the putatively toxic elements and the ameliorating machineries are generally neither tightly linked genetically nor necessarily reciprocally dependent (TEs may spread in the population despite efficient amelioration). However, some cases more closely mirror true toxin-antitoxin systems (Figure 1C). Ciliates undergo TE removal from the somatic genome, and the machinery responsible for TE removal is related to the TE mobilization machinery. This relationship suggests the possibility that genes encoding the machinery may have ensured their maintenance in the genome by creating insertions whose removal requires the machinery's maintenance. Similarly, multifunctional mobilization/splicing factors, such as maturases, encoded by type II self-splicing introns, may mobilize introns that do not encode the maturase, thus creating new insertions that require maintenance of the maturase gene. This last speculation could have been of particular importance during the massive fragmentation of nuclear genes in early eukaryotes by type II or associated introns [9].

In total, the above picture amounts to a third potential general explanation for the origins of complexity, adding to adaptationist and neutral perspectives. Whereas adaptationist perspectives emphasize selective benefits for the individual steps leading to complex phenomena, and a neutralist explanation emphasizes stochastic processes [2] and the possibility of 'constructive neutral evolution' [10], this third perspective recognizes a constructive role for selfish genes and intragenomic conflict. On this 'constructive conflict' model, conflict between elements of the same genome, particularly selfish elements and their suppressors, could drive increased complexity of regulatory logic, cellular machinery, and genome architecture. This

complexity may in turn serve as a substrate for the evolution of diverse novel functions. This perspective, which will clearly require further elaboration, may provide a promising source of explanation for diverse poorly-understood organismal and genetic phenomena.

The results of Li et al. expand the list of elements of CRISPR-Cas cassettes as well as the functions by which Cas shapes the genomes and transcriptomes of diverse prokaryotes. It will be fascinating to learn about the diversity of toxin-antitoxin systems associated with CRISPR-Cas as well as the broader diversity of mechanisms involved in the maintenance and innovation of cellular functions.

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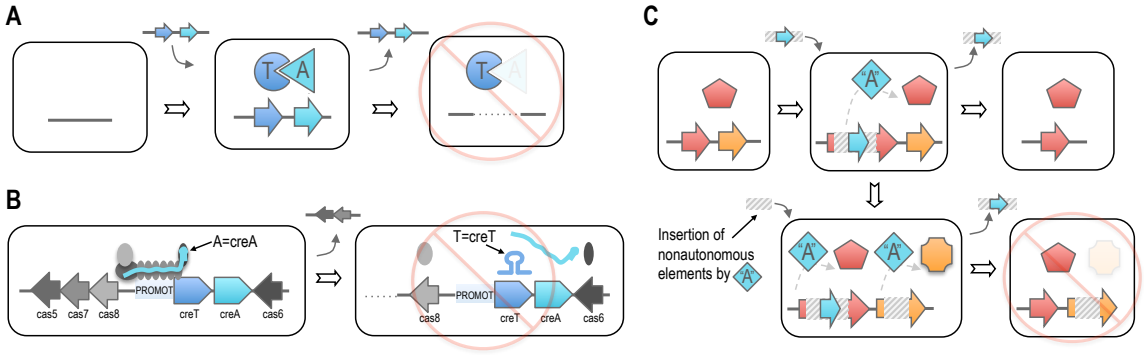

Figure 1. Classical, reported, and hypothesized toxin-antitoxin systems. A. Diagram of a classic toxin-antitoxin system, in which a toxin with a long half-life kills cells that lose the antitoxin-encoding gene. B. The novel CRISPR-Cas system reported by Li et al., in which the cas5-8 complex binds creA to repress the expression of the toxic creT gene. Loss of any of the cas genes or creA thus leads to toxicity. C. Proposed multi-locus system, involving a multifunctional TE-encoded gene with roles in both gene expression and TE mobilization. The TE-encoded gene is necessary for expression of TE-interrupted loci, for instance either by splicing at the RNA level or by splicing at the DNA level (as in ciliates). Thus, by mobilizing TEs that do not encode the gene ("nonautonomous" TEs"), the TE-encoded gene creates interrupted loci (orange) that require its function to be expressed. Thus, loss of the blue TE-encoded gene becomes toxic.