

= Toxin @-@ antitoxin system =

A toxin @-@ antitoxin system is a set of two or more closely linked genes that together encode both a protein 'poison' and a corresponding 'antidote'. When these systems are contained on plasmids ? transferable genetic elements ? they ensure that only the daughter cells that inherit the plasmid survive after cell division. If the plasmid is absent in a daughter cell, the unstable anti @-@ toxin is degraded and the stable toxic protein kills the new cell; this is known as 'post @-@ segregational killing' (PSK). Toxin @-@ antitoxin systems are widely distributed in prokaryotes, and organisms often have them in multiple copies.

Toxin @-@ antitoxin systems are typically classified according to how the antitoxin neutralises the toxin. In a type I toxin @-@ antitoxin system, the translation of messenger RNA (mRNA) that encodes the toxin is inhibited by the binding of a small non @-@ coding RNA antitoxin to the mRNA. The protein toxin in a type II system is inhibited post @-@ translationally by the binding of another protein antitoxin. A single example of a type III toxin @-@ antitoxin system has been described whereby a protein toxin is bound directly by an RNA molecule. Toxin @-@ antitoxin genes are often transferred through horizontal gene transfer and are associated with pathogenic bacteria, having been found on plasmids conferring antibiotic resistance and virulence.

Chromosomal toxin @-@ antitoxin systems also exist, some of which perform cell functions such as responding to stresses, causing cell cycle arrest and bringing about programmed cell death. In evolutionary terms, toxin @-@ antitoxin systems can be considered selfish DNA in that the purpose of the systems are to replicate, regardless of whether they benefit the host organism or not. Some have proposed adaptive theories to explain the evolution of toxin @-@ antitoxin systems; for example, chromosomal toxin @-@ antitoxin systems could have evolved to prevent the inheritance of large deletions of the host genome. Toxin @-@ antitoxin systems have several biotechnological applications, such as a method of maintaining plasmids in cell lines, targets for antibiotics, and as positive selection vectors.

= = Evolutionary advantage = =

Plasmid stabilising toxin @-@ antitoxin systems have been used as examples of selfish DNA as part of the gene centered view of evolution. It has been theorised that toxin @-@ antitoxin loci serve only to maintain their own DNA, at the expense of the host organism. Other theories propose the systems have evolved to increase the fitness of plasmids in competition with other plasmids. Thus, the toxin @-@ antitoxin system confers an advantage to the host DNA by eliminating competing plasmids in cell progeny. This theory was corroborated through computer modelling. This does not, however, explain the presence of toxin @-@ antitoxin systems on chromosomes.

Chromosomal toxin @-@ antitoxin systems have a number of adaptive theories explaining their success at natural selection. The simplest explanation of their existence on chromosomes is that they prevent harmful large deletions of the cell's genome, though arguably deletions of large coding regions are fatal to a daughter cell regardless. MazEF, a toxin @-@ antitoxin locus found in *E. coli* and other bacteria, induces programmed cell death in response to starvation, specifically a lack of amino acids. This releases the cell's contents for absorption by neighbouring cells, potentially preventing the death of close relatives, and thereby increasing the inclusive fitness of the cell that perished. This is an example of altruism and how bacterial colonies resemble multicellular organisms.

Another theory states that chromosomal toxin @-@ antitoxin systems are designed to be bacteriostatic rather than bactericidal. RelE, for example, is a global inhibitor of translation during nutrient stress, and its expression reduces the chance of starvation by lowering the cell's nutrient requirements. A homologue of mazF toxin called mazF @-@ mx is essential for fruiting body formation in *Myxococcus xanthus*. When nutrients become limiting in this swarming bacteria, a group of 50 @, @ 000 cells converge into a fruiting body structure. The maxF @-@ mx toxin is a component of this nutrient @-@ stress pathway; it enables a percentage of cells within the fruiting body to form myxospores. It has been suggested that *M. xanthus* has hijacked the toxin @-@

antitoxin system , replacing the antitoxin with its own molecular control to regulate its development .

It has also been proposed that chromosomal copies of plasmid toxin @-@ antitoxin systems may serve as anti @-@ addiction modules ? a method of omitting a plasmid from progeny without suffering the effects of the toxin . An example of this is an antitoxin on the *Erwinia chrysanthemi* genome that counteracts the toxic activity of an F plasmid toxin counterpart .

Nine possible functions of toxin @-@ antitoxin systems have been proposed . These are :

Junk ? they have been acquired from plasmids and retained due to their addictive nature .

Stabilisation of genomic parasites ? chromosomal remnants from transposons and bacteriophages .

Selfish alleles ? non @-@ addictive alleles are unable to replace addictive alleles during recombination but the opposite is able to occur .

Gene regulation ? some toxins act as a means of general repression of gene expression while others are more specific .

Growth control ? bacteriostatic toxins , as mentioned above , restrict growth rather than killing the host cell .

Persisters ? some bacterial populations contain a sub @-@ population of ' persisters ' controlled by toxin @-@ antitoxin systems that are slow @-@ growing , hardy individuals , which potentially insure the population against catastrophic loss .

Programmed cell arrest and the preservation of the commons ? the altruistic explanation as demonstrated by MazEF , detailed above .

Programmed cell death ? similar to the above function , although individuals must have variable stress survival level to prevent entire population destruction .

Antiphage mechanism ? when bacteriophage interrupt the host cell 's transcription and translation , a toxin @-@ antitoxin system may be activated that limits the phage 's replication .

An experiment where five TA systems were deleted from a strain of *E. coli* found no evidence that the TA systems conferred an advantage to the host . This result casts doubt on the growth control and programmed cell death hypotheses .

= = System types = =

= = = Type I = = =

Type I toxin @-@ antitoxin systems rely on the base @-@ pairing of complementary antitoxin RNA with the toxin 's mRNA . Translation of the mRNA is then inhibited either by degradation via RNase III or by occluding the Shine @-@ Dalgarno sequence or ribosome binding site . Often the toxin and antitoxin are encoded on opposite strands of DNA . The 5 ' or 3 ' overlapping region between the two genes is the area involved in complementary base @-@ pairing , usually with between 19 ? 23 contiguous base pairs .

Toxins of type I systems are small , hydrophobic proteins that confer toxicity by damaging cell membranes . Few intracellular targets of type I toxins have been identified , possibly due to the difficult nature of analysing proteins that are poisonous to their bacterial hosts .

Type I systems sometimes include a third component . In the case of the well @-@ characterised hok / sok system , in addition to the hok toxin and sok antitoxin , there is a third gene , called mok . This open reading frame almost entirely overlaps that of the toxin , and the translation of the toxin is dependent on the translation of this third component . Thus the binding of antitoxin to toxin is sometimes a simplification , and the antitoxin in fact binds a third RNA , which then affects toxin translation .

= = = Example systems = = =

= = = Type II = = =

Type II toxin @-@ antitoxin systems are generally better @-@ understood than type I. In this system a labile protein antitoxin tightly binds and inhibits the activity of a stable toxin . The largest family of type II toxin @-@ antitoxin systems is vapBC , which has been found through bioinformatics searches to represent between 37 and 42 % of all predicted type II loci .

Type II systems are organised in operons with the antitoxin protein typically being located upstream of the toxin . The antitoxin inhibits the toxin by downregulating its expression . The proteins are typically around 100 amino acids in length , and exhibit toxicity in a number of ways : CcdB protein , for example , affects DNA gyrase by poisoning DNA topoisomerase II whereas MazF protein is a toxic endoribonuclease that cleaves cellular mRNAs at specific sequence motifs . The most common toxic activity is the protein acting as an endonuclease , also known as an interferase .

A third protein can sometimes be involved in type II toxin @-@ antitoxin systems . In the case of the aforementioned MazEF addiction module , in addition to the toxin and antitoxin there is a regulatory protein involved called MazG . MazG protein interacts with E. coli 's Era GTPase and is described as a ' nucleoside triphosphate pyrophosphohydrolase , ' which hydrolyses nucleoside triphosphates to monophosphates . Later research showed that MazG is transcribed in the same polycistronic mRNA as MazE and MazF , and that MazG bound the MazF toxin to further inhibit its activity .

= = = Example systems = = =

= = = Type III = = =

Type III toxin @-@ antitoxin systems rely on direct interaction between a toxic protein and an RNA antitoxin . The toxic effects of the protein are neutralised by the RNA gene . One example is the ToxIN system from the bacterial plant pathogen *Erwinia carotovora* . The toxic ToxN protein is approximately 170 amino acids long and has been shown to be toxic to E. coli . The toxic activity of ToxN is inhibited by ToxI RNA , an RNA with 5 @.@ 5 direct repeats of a 36 nucleotide motif ( AGGTGATTTGCTACCTTTAAGTGCAGCTAGAAATTC ) . Crystallographic analysis of ToxIN has found that ToxN inhibition requires the formation of a trimeric ToxIN complex , whereby three ToxI monomers bind three ToxN monomers ; the complex is held together by extensive protein @-@ RNA interactions .

= = Biotechnological applications = =

The biotechnological applications of toxin @-@ antitoxin systems have begun to be realised by several biotechnology organisations . A primary usage is in maintaining plasmids in a large bacterial cell culture . In an experiment examining the effectiveness of the *hok / sok* locus , it was found that segregational stability of an inserted plasmid expressing beta @-@ galactosidase was increased by between 8 and 22 times compared to a control culture lacking a toxin @-@ antitoxin system . In large @-@ scale microorganism processes such as fermentation , progeny cells lacking the plasmid insert often have a higher fitness than those who inherit the plasmid and can outcompete the desirable microorganisms . A toxin @-@ antitoxin system maintains the plasmid thereby maintaining the efficiency of the industrial process .

Additionally , toxin @-@ antitoxin systems may be a future target for antibiotics . Inducing suicide modules against pathogens could help combat the growing problem of multi @-@ drug resistance .

Ensuring a plasmid accepts an insert is a common problem of DNA cloning . Toxin @-@ antitoxin systems can be used to positively select for only those cells that have taken up a plasmid containing the inserted gene of interest , screening out those that lack the inserted gene . An example of this application comes from CcdB @-@ encoded toxin , which has been incorporated into plasmid vectors . The gene of interest is then targeted to recombine into the CcdB locus , inactivating the transcription of the toxic protein . Thus , cells containing the plasmid but not the insert perish due to the toxic effects of CcdB protein , and only those that incorporate the insert survive .

Another example application involves both the CcdB toxin and CcdA antitoxin . CcdB is found in recombinant bacterial genomes and an inactivated version of CcdA is inserted into a linearised plasmid vector . A short extra sequence is added to the gene of interest that activates the antitoxin when the insertion occurs . This method ensures orientation @-@ specific gene insertion .

Genetically modified organisms must be contained in a pre @-@ defined area during research . Toxin @-@ antitoxin systems can cause cell suicide in certain conditions , such as a lack of a lab @-@ specific growth medium they would not encounter outside of the controlled laboratory set @-@ up .