Toxin-Antitoxin (TA) Systems in Stress Survival and Pathogenesis

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Abstract

Mycobacterium tuberculosis (*M.tb*), by virtue of its ability to evolve, has developed mechanisms that enable it to modulate its growth through regulation of replication, transcription, translation, generation of heterogeneous population of persister cells, etc. for survival in different stressful environment during its infection cycle. Toxin-antitoxin (TA) systems are ubiquitous in prokaryotic genomes that enable them to survive in various unfavourable conditions. A toxin protein may inhibit the growth, whereas an antitoxin may neutralize the effect of toxin in different ways. TA systems are involved in stress adaptation, antimicrobial tolerance or resistance, modification in the physiological state of organisms, biofilms formation, growth regulation for survival, plasmid maintenance, anti-phage activities, virulence, and programmed cell death.

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Environmental microorganisms express a wider repertoire of TA systems as compared to intracellular human pathogens due to a higher probability to encounter different environmental stresses within their ecosystem. However, the presence of high level of TA systems in *M.tb* is due to the fact that *M.tb* has to endure several types of stresses including acidic, hypoxic, oxidative, and immune surveillance within the host for its survival. TA systems are also present in pathogenic bacteria infecting plants. Based on the mechanism of action, different types of TA systems are classified within the microorganisms. Recently, genes related to type II TA systems have been proposed to be useful in genotyping of tuberculosis caused by different strains of *M.tb*.

Keywords
Toxin-antitoxin systems · *Mycobacterium tuberculosis* · Stress survival · Growth regulation · Drug tolerance

Abbreviations

ATP	Adenosine triphosphate
DATIN	Dormancy-associated translation inhibitor
DNA	Deoxyribonucleic acid
DR	Direct repeat
E. coli	*Escherichia coli*
IS	Insertion sequence
M.tb	*Mycobacterium tuberculosis*
mRNA	Messenger RNA
MTBC	*M.tb* complex
PCD	Programmed cell death
PSK	Post-segregational killing
SNPs	Single-nucleotide polymorphisms
sRNA	Small regulatory RNAs
TA	Toxin-antitoxin
TAC	Toxin-antitoxin-chaperone
Vap	Virulence-associated protein
VNTR	Variable number tandem repeats

15.1 Introduction

Toxin-antitoxin (TA) systems in bacteria were first recognized as plasmid-borne loci which help in plasmid maintenance through elimination of daughter cells lacking TA encoding plasmid (Guglielmini and Melderen 2011). A set of linked genes, together encoding a protein 'poison' and a corresponding 'antidote', forms the TA system (Gerdes 2000). The TA systems present on plasmids make sure that only the

daughter cells inheriting the plasmid survive after cell division. In daughter cells devoid of plasmid, unstable antitoxin is degraded while the stable toxic protein kills the new cell, and this phenomenon is known as 'post-segregational killing' (PSK). TA systems are present in multiple copies in prokaryotes (Yamaguchi et al. 2011). Various microbial genome analyses have comprehensively highlighted the diversity in the distribution of TA systems.

Previous studies have shown that the genomes of *Nitrosomonas europaea*, *Sinorhizobium meliloti*, and *Mycobacterium bovis* contain more than 50 presumptive TA systems, whereas *Rickettsia prowazekii*, *Bacillus subtilis, Campylobacter jejuni*, etc. contain no or very few TA systems (Pandey and Gerdes 2005; Sevin and Barloy-Hubler 2007). However, there is little consensus to prove correlation between the number of TA systems and the growth rate of the members within a phylum. Additionally, diversity in the distribution of TA systems among different isolates of the same species is also observed.

Detailed study of phylogenetic patterns of TA loci in several prokaryotic genomes suggests presence of multiple TA loci in free-living prokaryotes and few or no TA loci in obligate intracellular prokaryotes (Pandey and Gerdes 2005). TA loci are beneficial to organisms that confront stressful environment. TA systems, also referred to as junk, are considered to be constituents of plasmids and have been retained within the cells due to their addictive nature (Kroll et al. 2010). Some toxins act as general repressors of gene expression, while others are more specific in regulating gene expression (Engelberg-Kulka et al. 2006; Pimentel et al. 2005). Some TA systems that act as bacteriostatic toxins play a key role in growth regulation and may restrict growth rather than kill the host cell (Diago-Navarro et al. 2010). 'Persisters' are slow-growing population of cells, which can survive stress and later grow into actively dividing cells when the environment is favourable (Kussell et al. 2005). It has been demonstrated that an imbalance between the level of toxin and its antitoxin due to overexpression or mutations in either of them results in high persistence (Fridman et al. 2014; Korch and Hill 2006). Interruption of transcription and translation machinery of host cells due to bacteriophage may also trigger activation of TA system that in turn limits phage replication, termed as antiphage mechanism (Hazan and Engelberg-Kulka 2004).

M.tb faces different stresses in its pathogenesis and possesses several proteins such as two component systems, sigma factors, TA systems, acid response, halophilic proteins, etc. for its survival (Kumar et al. 2018). As compared to other mycobacteria, *M.tb* shows presence of a significant number of TA systems in its genome, which during the state of persistence are induced by active toxins, which may largely contribute towards its pathogenesis (Ramage et al. 2009). Transcriptomic analyses of antibiotic-induced *M.tb* persisters showed that about 10 TA systems were significantly upregulated, pointing to the importance of TA system in *M.tb* persistence (Keren et al. 2011). Interestingly, *M.tb* possesses abundant number of TA loci, while *M. leprae* has none, possibly due to the fact that *M. leprae* has evolved from *M.tb* through reductive evolution (Ramage et al. 2009; Cole et al. 2001). The presence of TA system provides an evolutionary edge to *M.tb* in terms of aiding its survival in both extra- and intracellular conditions as compared to *M. leprae* which can survive

only as obligate intracellular pathogen. Similarly, obligate intracellular microorganisms such as *Rickettsia* and *Buchnera spp*. have either very few or no TA loci, although several exceptions are also in existence (Pandey and Gerdes 2005; Leplae et al. 2011). Analyses of bacterial gene sequences by Shao et al. (Shao et al. 2011) conclusively point to the presence of TA systems in symbiotic bacteria and overrules previous studies by Pandey et al. that reported absence of TA systems in symbiotic bacteria (Pandey and Gerdes 2005; Shao et al. 2011).

15.2 Classes of Toxin-Antitoxin Systems

The TA loci are classified into different groups such as vapBC, parDE, relBE, ccd, phd/doc, mazEF, and higBA due of differences in mechanism of action (Gerdes et al. 2005). In *M.tb*, majority of TA systems belong to the class of VapBC (virulence-associated protein) (Gerdes and Maisonneuve 2012). VapC induces dormancy by suppressing translation and induction of vapB transcription which later leads to revival of cells (Winther and Gerdes 2009). It has been shown recently by deletion and overexpression studies that some members of the VapBC TA systems of *M.tb* are involved in bacteriostasis, morphological changes, growth arrest, and mycobacterial pathogenesis (Agarwal et al. 2018). *M.tb* VapBC30 system has been shown to be involved in growth regulation through ribonuclease activity (Deep et al. 2018). *M. tb* toxin VapC30 inhibits the growth of *Escherichia coli (E. coli)* when expressed without its cognate antitoxin VapB30. There is no effect of *M.tb* VapC30 on *E. coli* when co-expressed with *M.tb* VapB30. *M.tb* VapC30 degrades RNA molecules that are magnesium and manganese ion dependent (Lee et al. 2015).

M.tb MazF toxin members, when expressed in *E. coli* or *Mycobacterium smegmatis,* affect their growth (Gupta 2009; Zhu et al. 2006). The overexpression of MazF3, MazF6, and MazF9 of *M.tb* in *Mycobacterium bovis BCG* induces bacteriostasis (Tiwari et al. 2015). *M.tb* MazF toxins are also involved in the drug tolerance, virulence, and stress adaptation (Tiwari et al. 2015).

The RelBE system is among the most characterized TA systems that bind with the A site of the ribosome and affect protein synthesis by cleaving the mRNAs preferably between the second and third nucleotides of the termination codon (Pedersen 2003). In contrast, RelE binds to initial coding region and cleaves the first 100 codons of mRNA and inhibits growth (Hurley et al. 2011). Variety of stress conditions such as nitrosative stress, oxidative stress, and antibiotic stress affect the transcript profiles of RelE toxins of *M.tb*. The overexpression of toxin RelE of *M.tb* affected growth of the *E. coli* and *M.tb.* The three RelE toxins of *M.tb* are involved in individual antibiotic specific tolerance (Singh et al. 2010).

The YefM antitoxin is highly unstable as it is prone to degradation by Lon, an ATP-dependent serine protease. YefM is co-expressed with the YoeB toxin and the resultant complex so formed consists of dimer of YefM and single molecule of YoeB (Kamada and Hanaoka 2005). Rv3357–Rv3358 of *M.tb* codes for YefM/YoeB system.

The HigBA family, HigB toxin (Rv1955) and HigA antitoxin (Rv1956), are part of the operon comprising of Rv1954A and Rv1957. Rv1957 is found as a SecB-like chaperone required for antitoxin stabilization. HigB inhibits protein synthesis by cleaving mRNAs that are being translated in *E. coli* (Smollett et al. 2009; Fivian-Hughes and Davis 2010; Christensen-Dalsgaard et al. 2010; Bordes et al. 2011).

The tripartite toxin-antitoxin-chaperone system (TAC) complex is induced during heat shock, hypoxia, nutrient starvation, and persistence. Within the TAC complex, the chaperone directly binds to HigA antitoxin and prevents it aggregation or degradation, thereby aiding in HigA folding and successive interaction with HigB toxin (Bordes et al. 2011).

M.tbH_{37}Rv also possesses two ParDE systems, and ParDE2 operon has been investigated recently and was found that toxin MParE2 interacts with GyrB subunit and inhibits bacterial growth by inhibiting DNA gyrase, thereby blocking DNA replication (Gupta et al. 2016).

15.3 Types of Toxin-Antitoxin Systems

TA system is characterized by neutralization of toxin by the antitoxin (Fig. 15.1). In case of a type I TA system, translation of messenger RNA encoding the toxin is inhibited by binding of a non-coding RNA antitoxin to the mRNA. The protein toxin in case of type II TA system is inhibited post-translationally through binding of another protein antitoxin. In case of type III TA systems, a small RNA binds directly to the toxin protein. Type IV-VI TA systems are also reported but are relatively less common. Toxin-antitoxin genes, transferred predominantly through horizontal gene transfer, are mostly associated with pathogenic bacteria and with plasmids conferring antibiotic resistance or virulence (Mine et al. 2009; Van Melderen and Saavedra De Bast 2009).

15.3.1 Type I TA System

In this type of TA system, antitoxins consist of small regulatory RNAs (sRNA) comprising of 50–200 nucleotides. A non-coding RNA antitoxin complementarily binds to the toxin-encoding mRNA resulting in mRNA degradation or inhibition of toxin translation (Brielle et al. 2016; Brantl and Jahn 2015). Type I toxin and antitoxins are transcribed from their own promoter, while in other types of TA systems, they are part of operon with other genes. The translation of the type I toxin mRNA is inhibited by base pairing with the antitoxin sRNA that prevents interaction with ribosome resulting in inhibition of translation of toxin mRNA (Brantl 2012; Fozo et al. 2008). Most of the type I toxins are small hydrophobic proteins that create pores in the inner membrane leading to breakdown of membrane potential to stop ATP synthesis, thereby blocking energy-demanding activities such as protein synthesis (Wen et al. 2014; Lee and Lee 2016). *sym*R/*sym*E module of *E. coli* is considered as an example of type I TA system (Kawano et al. 2007).

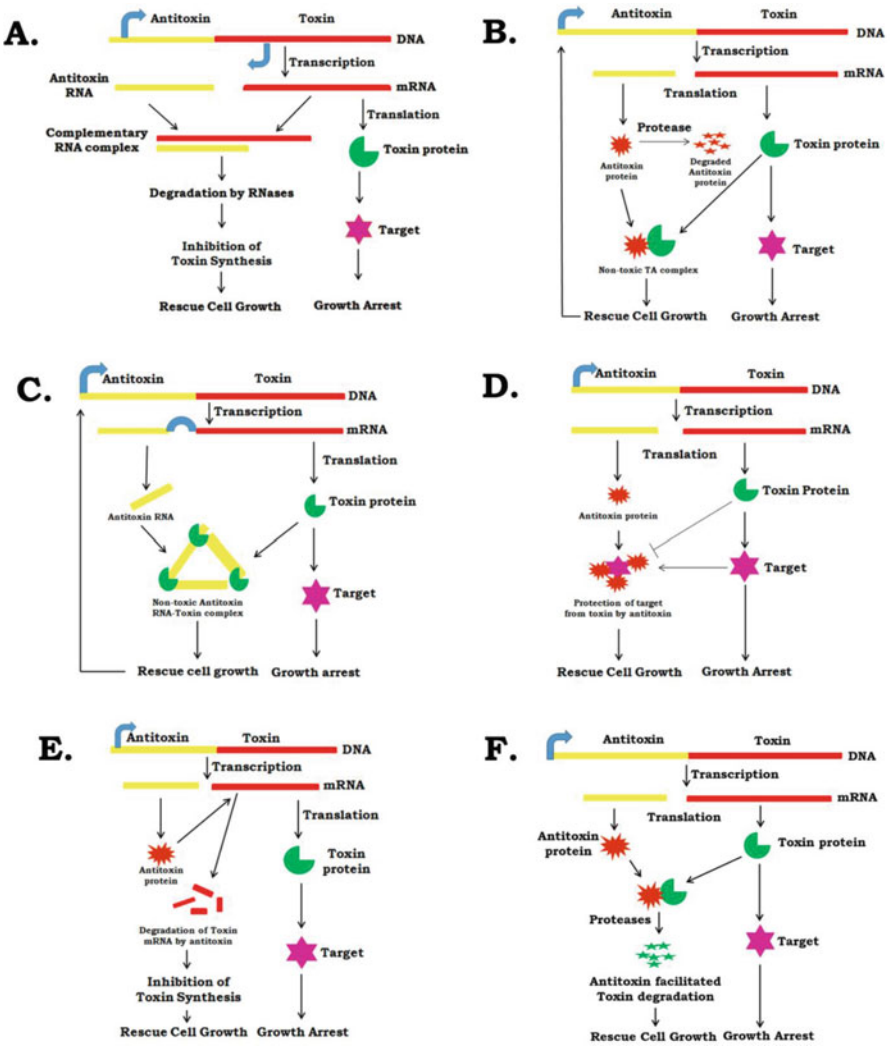

Fig. 15.1 Types of toxin-antitoxin systems: Toxin proteins inhibit the growth of cells and there are different mechanisms through which antitoxins neutralize the effect of toxins: (**a**) Suppression in translation of toxins by complementary RNA complex formation between toxin and antitoxin mRNA. (**b**) Non-functional toxin-antitoxin protein complex neutralizes the effect of toxins on growth. (**c**) Non-functional complex formation due to interaction of antitoxin mRNA with toxin proteins. (**d**) Interaction of antitoxin with target proteins stops toxin activities. (**e**) Antitoxin protein degrades mRNA of toxin resulting in suppression of toxin protein synthesis. (**f**) Antitoxins facilitate degradation of toxins by proteinases resulting in rescue of cell growth

15.3.2 Type II TA System

Type II TA systems are most extensively characterized in both prokaryotes and archaea. In type II TA system, the functional activity of toxin proteins is inhibited due to interaction between stable toxin and labile antitoxin. Toxin and antitoxin proteins are expressed simultaneously as both are organized into operons. The activity of different toxin protein regulates various mechanisms within the cell. For example, CcdB protein of *E. coli* (strain K12) inhibit the function of DNA gyrase by inactivating DNA topoisomerase II, whereas MazF cleaves cellular mRNAs for the inhibition of protein synthesis (Bernard and Couturier 1992; Zhang et al. 2003). The TA complex acts as a repressor for TA operon system as it binds to the palindromic sequence of the promoter region. Due to antitoxin degradation, the concentration of TA complex reduces thereby leading to production of more toxins and antitoxin.

15.3.3 Type III TA System

Type III TA system is characterized by direct interaction between a toxic protein and RNA antitoxin. The RNA gene involved totally neutralizes the effects of the toxic protein. ToxI-ToxN TA system from a plant pathogen named *Erwinia carotovora* subspecies *atrosepticum* (*Pectobacterium atrosepticum*) is a perfect example of type III TA system. The function of toxin protein (ToxN) is directly suppressed by interaction with antitoxin RNA, forming aToxN-RNA (an RNA antitoxin) complex (Fineran et al. 2009; Blower et al. 2012).

15.3.4 Type IV TA System

In type IV TA system, there is no direct interaction between toxin and antitoxin proteins. Antitoxin protein interacts with the target of toxin protein, thereby suppressing the activity of toxin on its target. The functional aspect of type IV TA system is exemplified in case of toxin CbeA of *E. coli* (1303) that prevents polymerization of the cytoskeletal proteins (MreB and FtsZ) and inhibits cell division. The antitoxin CbeA (YeeU) protein inhibits by binding directly with the target, namely, MreB and FtsZ toxin, instead of forming a toxin-antitoxin complex (Masuda et al. 2012).

15.3.5 Type V TA System

In the type V TA system, antitoxin protein retards synthesis of toxin protein by degrading the mRNA transcribed to code toxin protein. This is exemplified by GhoT

of *E. coli* (strain K12), a protein that induces persistence and ghost cell formation with damaged membrane. The antitoxin GhoS is an endoribonuclease and precisely cleaves the mRNA encoding for membrane-lytic peptide toxin GhoT (Wang et al. 2013).

15.3.6 Type VI TA System

In the type VI TA system, antitoxin protein facilitates the proteolytic degradation of toxin protein. There is no degradation of toxin protein by proteases in the absence of antitoxin, and if toxin-antitoxin proteins are together, toxin degradation occurs. In SocAB TA system in *Caulobacter crescentus*, SocB protein inhibits DNA elongation by intercalating with DnaN, whereas antitoxin SocA facilitates degradation of SocB in the presence of protease ClpXP (Aakre et al. 2013).

15.4 Biological Roles of Toxin-Antitoxin Systems

TA systems regulate bacterial survival in different types of unfavourable conditions. TA systems are involved in several biological functions such as growth regulation, physiological changes of the cells, programmed cell death, etc. (Fig. 15.2). A more detailed importance of the TA systems in various physiological conditions is described below:

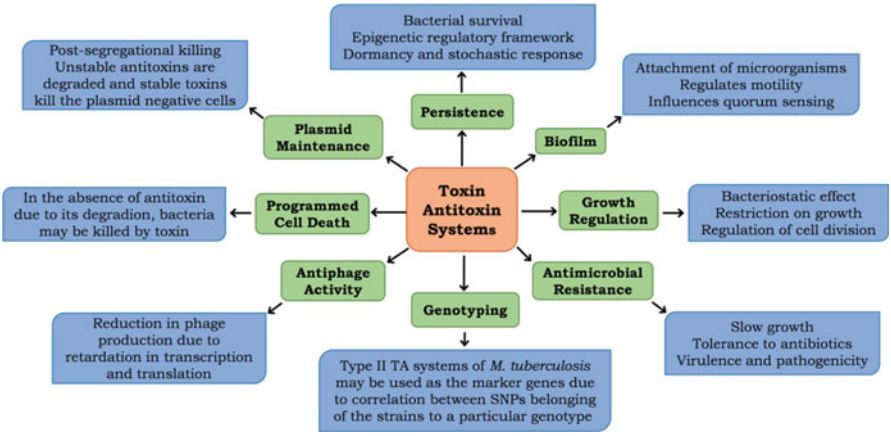

Fig. 15.2 Roles and applications of toxin-antitoxin systems: Toxin-antitoxin systems play important roles such as plasmid maintenance, drug resistance/tolerance, biofilm formation, growth regulation, antiphage activity, genotyping, programmed cell death, etc.

15.4.1 Stress Survival

TA systems act on regulatory machinery that control mechanisms critical for survival of bacteria (Engelberg-Kulka et al. 2006; Yamaguchi et al. 2011). For example, TA systems are important for adaptation of *M.tb* to unfavourable environmental conditions inside the host and maybe required for triggering a non-replicating state (Lewis 2007). Mycobacteria often adopt a non-replicative persistent, inactive state, to avoid unfavourable stress conditions like hypoxia, oxidative stress, nutritional limitations, acidic pH, etc., within the host macrophages (Wu et al. 2012). There are 88 putative TA systems present in *M.tb*H_{37}Rv that are conserved in *M.tb* complex (MTBC) but are few in other non-pathogenic mycobacteria, indicating a potential contribution to the pathogenic lifestyle of *M.tb* (Ramage et al. 2009). In response to starvation, several toxins within the cells are upregulated leading to inhibition of translation and selective degradation of mRNA. Contrary to starvation which leads to generalized upregulation of TA loci, low pH exposure leads to downregulation of few TA genes (Gupta et al. 2017).

15.4.2 Persistence

In majority of bacteria, there are set of genes that are involved in growth inhibition, and their overexpression may result in cell death, similar to programmed cell death of eukaryotes (Wen et al. 2014). It has been reported that TA systems are important for persister phenotype in *E. coli* (Tsilibaris et al. 2007). TA systems present in *M.tb* may regulate cell division during infection (Warner and Mizrahi 2006). After infection, *M.tb* initially grows and then acquires latency, a state of non-replicating cells in unfavourable conditions that can survive long periods with the potential to reactivate itself later whenever environment is favourable (Stewart et al. 2003, North and Jung 2004). In the case of persistence, a subpopulation of non-replicating bacteria becomes tolerant to antibiotics (Gomez and McKinney 2004). The presence of TA system in *M.tb*, as in other bacteria, imparts antibiotic tolerance and is one of the main reasons why long term of antibiotic therapy is required to cure tuberculosis (Keren et al. 2004; Ramage et al. 2009). It has been reported that mycobacterial MazF ribonucleases are involved in the drug tolerance, adaptation in oxidative stress, and nutrient depletion and virulence (Tiwari et al. 2015). *M.tb* RelE toxins are involved in formation of persisters specific to individual antibiotic and involved in drug-specific tolerance (Singh et al. 2010).

15.4.3 Biofilms

Planktonic bacteria can aggregate and attach themselves on biotic or abiotic surfaces to form biofilms. Formation of biofilms by pathogens is considered to be one of the

main survival strategies to counter the host defence (Rybtke et al. 2011; Kumar et al. 2017a). TA systems have been shown to be involved in biofilm formation, with exceptions. It has been shown that type II TA system of *E. coli* involving MqsR protein is induced during biofilm formation and deletion of this gene resulted in the absence of biofilm formation (Kasari et al. 2010). The role of m*qsRA* TA system in biofilm formation is associated with motility and as autoinducer-2 quorum sensing system (Gonzalez Barrios et al. 2006). The yefM-yoeB and relBE TA systems of *Streptococcus pneumonia* are involved in the biofilm formation. It has been reported that mutant strains lacking yefM-yoeB or both yefM-yoeB and relBE show reduction in biofilm formation (Chan et al. 2018). It has also been shown that deletion of mutants of *mazF* and *relE* gene homologue did not affect biofilm formation in *Streptococcus mutans* (Lemos et al. 2005).

15.4.4 Antiphage Activity

The competitive edge of pathogen against the host is driven by the enormous diversity and multiplicity of TA systems. Bacteria have evolved several defence mechanisms to protect themselves and survive against the onslaught of phage infection. Bacteria exhibit a wide array of mechanisms to resist bacteriophages that include inhibition of adsorption, exclusion of superinfection, cleavage of nucleic acids of the phages through restriction-modification or CRISPR-Cas systems, and abortive infection (Stern and Sorek 2012; Seed 2015). The abortive infection systems trigger premature death of phage-infected bacteria and restrict the phage to replicate or spread, thereby protecting the uninfected bacterial population within the niche. Thus, there is a link between abortive infection and TA systems. Several TA systems have anti-phage activity including Hok-Sok, LsoAB and MazEF, ToxIN, and AbiEG (Short et al. 2018).

15.4.5 Bacterial Virulence and Pathogenecity

Presence of TA modules in the genome is directly related to the virulence of bacteria (Georgiades and Raoult 2011), for example, type I toxins are involved in lysis of host cell. The PepA1 toxin of *S. aureus* is a pore-forming peptide that causes bacterial cell death. When there is oxidative stress inside host cell, the PepA1 toxin is typically released from its SprA antitoxin. This is an example of altruistic behaviour, as the peptide drives erythrocyte lysis, resulting in release of slowly dividing cells that escape the immune system (Sayed et al. 2012). Deletion of VapBC homologues in *Haemophilus influenzae* results in remarkable decrease of virulence in animal models for otitis media, tissue, etc. (Ren et al. 2012).

15.4.6 Growth Regulation

M.tb encounters different types of unfavourable conditions during infection. To survive in stressful conditions, bacteria regulate its growth. There are several proteins that are involved in growth regulation other than TA systems such as DATIN, IciA, MSMEG_1878 (*M.tb*Rv3241c orthologue in *M. smegmatis*), etc. *M. tb* DATIN and MSMEG_1878 inhibit protein synthesis by interacting with ribosome (Kumar et al. 2012; Li et al. 2018). *M.tb* IciA inhibits bacterial growth by inhibiting the opening of two strands of DNA during replication (Kumar et al. 2009; Kumar et al. 2017b). Toxin-antitoxin systems are activated during starvation stress. The RNase toxins, instead of being bactericidal, are usually bacteriostatic in nature. There is quick arrest of growth in response to starvation or other environmental stresses which help in their survival, and quicker resumption of growth occurs when the situation improves (Gerdes 2000; Gerdes et al. 2005).

15.4.7 Programmed Cell Death

Programmed cell death (PCD or apoptosis) is a physiological process and occurs mainly in multicellular, eukaryotic organisms during the process of embryonic development or tissue turnover. Dysregulation of PCD results in diseases like tumour formation, autoimmune diseases, or lysosomal disorders (Hayes 2003). Bacteria, being unicellular, do undergo PCD. However in natural environment, bacteria exist as biofilms that represent multicellular colonies and display coordination as in multicellular organisms. Such immobilized bacteria maintain discrete and ordered spatial structures within the biofilm niche. There are several genes in bacteria that are homologues to eukaryotic genes involved in PCD (Koonin and Aravind 2002), and TA modules of *E. coli* are either PCD genes or mediators of reversible growth arrest, which alternatively might allow the cells to enter a dormant or a semi-dormant state. It has been shown that PCD in bacteria might allow surviving cells to scavenge nutrients from dead ones and may prevent spread of bacteriophages (Engelberg-Kulka and Glaser 1999).

15.5 Applications of Toxin-Antitoxin Systems

There is a growing need for new antimicrobial agents due to decrease in the effectivity of drugs being used currently arising out of increase in multi-drug resistance. The toxins of TA systems usually target various biological processes such as replication, transcription, translation, macromolecular synthesis, cell wall synthesis, phage infection, and cytoskeletal polymerization. The fact that some of these toxins also overlap with the targets of the antibiotics (Wen et al. 2014) provides an option to explore TA systems as drug target in bacteria. There are several antibiotics that act indirectly against the TA systems. The detailed study of the interaction between the toxin and the antitoxin may help in the formulation of new

drugs. Mapping the precise location of different TA systems in bacterial chromosome or plasmid will uncover fundamental insight into their possible applications as drug targets.

Previously, IS-elements, DR-elements, variable number tandem repeats (VNTR), and single-nucleotide polymorphisms (SNPs) were used as genetic markers in housekeeping genes or other genes and were used for genotyping. The type II TA systems are involved in virulence, persistence, and survival of *M.tb* inside host macrophages (Zaychikova et al. 2015). Analyses of 173 sequenced genomes of *M. tb* for the genes of type II TA systems show genetic diversity (SNPs) that correlates with the specific genotype of *M.tb* strains (Zaychikova et al. 2015). This correlation between a genotype of particular strain and SNPs in different genes of type II TA system paved way to consider TA systems as a new biomarker for genotyping of tuberculosis caused by different strains of *M.tb.*

15.6 Conclusion

Genome sequencing and its annotation have suggested presence of significant number of TA systems in microorganisms. The classification of TA system is based on similarity of primary sequences and on the specificity of interaction between pair of toxin and antitoxin molecules. Type II system is structurally most flexible in terms of location of antitoxin gene either upstream or downstream of the toxin gene or regulation of repression activities that is encoded by different genes. The various types of TA system may shuffle among themselves in terms of function and can exhibit 'mix and match' phenomenon. This is exemplified by the structural similarity between GinI solitary toxins with type II HicA toxin. In some cases, toxins can also evolve from the antitoxin under selective pressure. As in the case of VapD sequences, which mediate defence against phages, TA system points to a common origin with CRISPR-cas system. There is consensus that the evolution of TA system in bacteria enabled these unicellular organisms into robust molecular machines that could withstand the onslaught of environment and various stress. The importance of TA system is also underlined by the fact that while many pathogenic and obligatory intracellular pathogens opted for reductive evolution in genome size and shed off the extra burden of many genes, yet they retained the genes associated with TA system. The distribution of TA system varies even among strains of the same species of bacteria. TA systems are highly mobile in nature and moves between genomes through horizontal gene transfer. The presence of TA genes within the transposons makes them refractory to gene efflux and stabilizes the TA system within the cells. The additive property of TA genes within the transposons facilitates increased stability and exclusion of foreign DNA. Although the activity of TA system may be subdued by host cell machinery, it may rescue its activity similar to the restriction modification system. TA systems are preferentially associated with genomic islands or plasmids which serve as a mechanism to maintain their structural integrity during stress and survival through post-segregational killing. *M.tb* possesses a huge number of TA systems that are usually located within distinct genomic islands and trigger

decrease in metabolic activity. In case the TA genes are integrated within the core genome, these accumulate mutation that may lead to loss of addictive property or deletion of TA system. In case of *E. coli*, type I *hok-sok* system are inactivated by IS sequences, gene rearrangements, and point mutations. TA system can evolve through integration with the host regulatory machinery and may replace the antitoxin molecules with signal transduction molecules.

Toxins have many cellular functions including inhibition of protein synthesis, DNA replication, and synthesis of cell wall in response to unfavourable conditions. Some toxins in TA system act as ribonucleases, while some other toxins act as gyrase inhibitors and kinases. The TA genes express in different stressed conditions such as nutrient deficiency, antibiotic treatment, bacteriophage infection, host immune responses, oxidative stress, and high temperature. They are involved in persistence, slow cell growth, cell cycle arrest, or cell death. Proteins involved in TA systems may act as important targets for drug development that may help in reduction of treatment duration of tuberculosis and other infectious diseases. In spite of the diversity in structure of TA system, the function of the TA system is tightly regulated by other cellular networks such that the prolific activity of TA system is activated only as a response to cellular physiology and the toxins are unleashed for minimal activity. The activity of TA locus that regulate activation of signaling pathways involved in persister cell formation in biofilms are modules acting as effectors of persister cell formation. These are usually deeply integrated into cellular signaling pathways that tightly control their activation and use their characteristic auto-regulatory features to tune the induction, duration, and intensity of the phenotypic switch into dormancy.

It is speculated that our current understanding of the toxin-antitoxin system is still redundant and a deeper understanding of the mechanistic significance of TA system will enable us to unravel the mysteries of how these unicellular organisms could dominate the environment. It is envisaged that unlocking the mechanism of TA system could allow us to shape better strategies for overcoming the harmful effects of TA system in clinical pathogenesis and in dealing with microbial drug tolerance.

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