BBA - Molecular Cell Research 1873 (2026) 120097

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Research paper

RelK, a YoeB-like toxin from *Mycobacterium tuberculosis* displays ribosome independent endonucleolytic activity

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ARTICLE INFO

Keywords:
Mycobacterium tuberculosis
Toxin-antitoxin modules
Persistence
Nuclease activity
Ser/Thr phosphorylation

ABSTRACT

Type II toxin-antitoxin (TA) modules are paired genetic elements encoding a toxin protein and an associated antitoxin that neutralizes the toxin under favourable growth conditions. Stress-induced degradation of the antitoxin renders the toxin free to disrupt essential cellular processes leading to growth arrest or cell death. *Mycobacterium tuberculosis (M. tb)* is known to harbor 60+ such modules that contribute to its pathogenicity and persistence. The RelK toxin of the RelJK cassette has generated significant interest given its expression during infection and regulation by post-translational modification; however, its functional activity remains uncharacterized. Using a cell free transcription-translation system we show that both, unphosphorylated and phosphorylated RelK toxin inhibit *in vitro* protein synthesis implicating a role in translational control, one that is enhanced by S/T phosphorylation. A key finding of this study is the ribosome-independent nuclease activity of RelK. We discovered that in addition to the *in vitro* RNase activity, RelK also cleaves double-stranded DNA in a dose-dependent manner, and is inactivated by the RelJ antitoxin. Further characterization established RelK as a nickase whose activity is modulated by divalent cations, and is independent of the substrate topology, although supercoiled DNA substrates are preferred. Molecular docking of RelK revealed multiple contacts with the phosphate backbone and bases of the dsDNA facilitating binding and protein orientation on the major groove region. Furthermore, substituting His84 with glutamine in RelK not only abolished its catalytic activity but also obliterated cytotoxicity. The data highlights the diversity in substrates and catalytic activities of *M. tb* Type II toxins.

1. Introduction

The evolution of *Mycobacterium tuberculosis (M. tb)*, an intracellular pathogen causing tuberculosis (TB) is remarkable as it continues to be among the leading causes of human mortality [1]. Upon infection, the bacillus either causes active disease or transitions into a metabolically inactive dormant/persistent state resulting in latent TB infection (LTBI) [2]. Being asymptomatic, LTB infections serve as reservoirs that can progress to reactivation of the TB disease following immune suppression caused by re-exposure, secondary infections or ageing. Understanding how mycobacteria transition into a state of persistence, maintain it and finally exit it, is imperative to develop diagnostic tools and therapeutics to control TB.

Toxin-antitoxin (TA) modules are highly prevalent in all prokaryotes with the genome of *M. tb* encoding more than 60 TA modules [3,4]. These modules play crucial roles in bacterial survival, persistence, stress-adaptation, tolerance to antibiotics, biofilm formation and pathogenesis [3–7] and hence are an important focus area in tuberculosis research. There are several types of TA modules (Types I-VIII) [8,9], with the Type II system being the most extensively studied. Typically, these modules are organized as two-gene operons, each with distinct structures, mechanisms, and evolutionary histories [10]. Degradation of the antitoxin releases the toxin to exert negative effects on growth, which otherwise would be sequestered by binding to the antitoxin under normal physiological conditions. Given the widespread occurrence and effects on cell survival and cell death, TA modules must be tightly regulated to prevent spurious induction of persistence. Autoregulation of the TA operon [11,12], antisense RNA [13], phosphorylation of the

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https://doi.org/10.1016/j.bbamcr.2025.120097
Received 19 August 2025; Received in revised form 13 December 2025; Accepted 16 December 2025
Available online 19 December 2025