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Role of *vapBC* toxin–antitoxin loci in the thermal stress response of *Sulfolobus solfataricus*

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Abstract

TA (toxin–antitoxin) loci are ubiquitous in prokaryotic microorganisms, including archaea, yet their physiological function is largely unknown. For example, preliminary reports have suggested that TA loci are microbial stress-response elements, although it was recently shown that knocking out all known chromosomally located TA loci in *Escherichia coli* did not have an impact on survival under certain types of stress. The hyperthermophilic crenarchaeon *Sulfolobus solfataricus* encodes at least 26 *vapBC* (where *vap* is virulence-associated protein) family TA loci in its genome. VapCs are PIN (PilT N-terminus) domain proteins with putative ribonuclease activity, while VapBs are proteolytically labile proteins, which purportedly function to silence VapCs when associated as a cognate pair. Global transcriptional analysis of *S. solfataricus* heat-shock-response dynamics (temperature shift from 80 to 90°C) revealed that several *vapBC* genes were triggered by the thermal shift, suggesting a role in heat-shock-response. Indeed, knocking out a specific *vapBC* locus in *S. solfataricus* substantially changed the transcriptome and, in one case, rendered the crenarchaeon heat-shock-labile. These findings indicate that more work needs to be done to determine the role of VapBCs in *S. solfataricus* and other thermophilic archaea, especially with respect to post-transcriptional regulation.

Keywords

archaeon; heat shock; hyperthermophile; stress response; *Sulfolobus solfataricus*; toxin–antitoxin locus

Introduction

TA (toxin–antitoxin) loci, also known as plasmid addiction or poison–antidote systems, were first identified as a plasmid maintenance mechanism that activated PSK (post-segregational killing) in plasmid-free progeny [1]. These loci encode a cognate protein pair, consisting of a proteolytically labile antitoxin and a toxin [2,3]. Available genome sequence data indicate that TA loci are also chromosomally encoded and ubiquitous in free-living prokaryotes [4]. The widespread occurrence of chromosomally encoded TA loci in the microbial world suggests an important function, although the role of these proteins in either a specific or general sense is largely unknown.

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TA loci are typically arranged in operons with the antitoxin gene preceding the toxin gene (except for *higBA* and *hipBA* where the toxin precedes the antitoxin) [5–7]. The TA ORFs (open reading frames) often overlap, making co-expression likely. The functional relationship between toxin and antitoxin proteins appears to be consistent across microbiology, although there is still only limited experimental evidence along these lines. As long as the antitoxin is present, it interacts with the cognate toxin to presumably neutralize toxic activity, which at least in some cases is ribonucleolytic [8–14]. However, when the antitoxins are lost in plasmid-free progeny or proteolytically degraded in chromosomally encoded cases, the free toxins are activated. The details of TA function are unique to specific types. For example, in some cases, TA operons are apparently regulated by the binding of the N-terminus of the antitoxin to the locus promoter region [15,16]. Toxins may also function as co-repressors, since their binding to the antitoxin appears to increase the affinity of the latter for the promoter region [17].

The precise function of chromosomally encoded TA loci remains controversial. TA loci may be bacteriocidal [18] or bacteriostatic [19,20]. In fact, *hipBA* TA loci have been linked to persister cell formation [21]. In *Escherichia coli* K-12, knocking out all five of the known chromosomally encoded TA loci did not have a significant impact on the survival of pH, nutritional or antibiotic stress [22]. Chromosomally encoded TA loci may act as anti-addiction modules that protect cells from PSK by plasmid-encoded TA loci [23]. Whereas TA loci in mesophilic bacteria have been closely examined, this is not the case in the archaea. Many archaeal genomes, particularly thermophilic archaea, encode multiple TA loci. The significance of these TAs under normal or abnormal growth conditions remains to be seen.

vapBC TA loci and archaea

The distribution of members of eight TA families in prokaryotic genomes is widespread. Out of 218 prokaryotic genomes surveyed, over 1472 TA loci have been identified with an additional 63 solitary toxins or antitoxins found [4,24]. To date, the *vapBC* (where *vap* is virulence-associated protein) family is the most abundant TA system among prokaryotic genomes, representing ~40% of all TA loci [4]. It is present in high numbers in the archaea, especially in hyperthermophiles and extreme thermoacidophiles (Figure 1).

Most VapB antitoxins contain an SpoVT/AbrB DNA-binding domain and, as such, belong to the superfamily of transcriptional regulators of the same name. AbrB, which has been studied extensively in *Bacillus subtilis* and *Bacillus anthracis*, is a transition-state regulator [25–27]. SpoVT, an AbrB homologue, was shown to regulate expression of at least 15 genes, probably via DNA-binding interactions with target promoters [28]. VapC toxins are characterized by a PIN (Pil-T N-terminus) domain (a domain homologous with the N-terminal domain of the pilin biogenesis protein Pil-T) [29]. In eukaryotes, PIN domain proteins are ribonucleases involved in nonsense-mediated mRNA decay and RNAi (RNA interference) [30]. PIN domains could provide clues to the cellular targets of VapC toxins, but this connection has yet to be made experimentally. Generally, VapCs are putative ribonucleases, although their precise specificity is not clear. For example, they were found to have endonuclease activity in mycobacteria and exonuclease activity in the hyperthermophilic crenarchaeon *Pyrobaculum aerophilum* [8,9]. Furthermore, a VapC from *Haemophilus influenzae* was determined to be ribonucleolytic, degrading free RNA *in vitro* [11].

Heat-shock-response of hyperthermophilic archaea

Even though extremely thermophilic archaea thrive at extreme temperatures, they also have thermal limits and display a classic heat-shock-response when thermally stressed [31–38].

This response involves the thermosome, or rosettasome, a heat-shock-responsive HSP (heat-shock protein) 60-like molecular chaperone that has been implicated in many cellular roles [39,40]. Examination of several hyperthermophilic archaea undergoing thermal stress has revealed that heat shock has a profound effect on the transcriptome. *Pyrococcus furiosus* response to a temperature shift from 90 to 105°C, included up-regulation of the thermosome (KEGG accession number PF1974) and a HSP20-like sHSP (small heat-shock protein) (accession number PF1883) [35], in addition to several hundred ORFs (K.R. Shockley and R.M. Kelly, unpublished work). Detailed analysis revealed that a novel heat-shock-regulator protein, Phr, in *P. furiosus* prevented synthesis of HSP20, AAA+ (ATPase associated with various cellular activities) (whose function is unclear) and itself by binding to the promoter regions and blocking the RNA polymerase-binding site when under both thermal stress as well as during nutrient-limited stationary growth phase [38]. The heat-shock-response of other hyperthermophilic archaea has also been examined. A temperature shift from 85 to 95°C was lethal for *Methanococcus jannaschii*; after 20 min at 95°C, 76 genes were up-regulated significantly (>2-fold), including an sHSP (KEGG accession number MJ0285) and the thermosome (KEGG accession number MJ0999) [31]. In *Archaeoglobus fulgidus*, 10% of the genome was differentially transcribed after only 5 min at 89°C (up from the normal growth temperature of 78°C); up-regulated ORFs included six of 13 known heat-shock-related genes (KEGG accession numbers AF1296, AF1297, AF1298, AF1451, AF2238 and AF1971). After 1 h at 89°C, 14% of the *A. fulgidus* genome displayed changes in mRNA transcription levels [34]. Among the hyperthermophilic archaea examined for heat-shock-response, *Sulfolobus solfataricus* exhibited the most pronounced change in transcriptome, with approx. one-third of its genome responding to a shift from 80 to 90°C within 5 min of reaching the target temperature; 37% of the up-regulated genes were insertion sequences. Both HSP20 family sHSPs (KEGG accession numbers SSO2427 and SSO2603) were up-regulated, as were many of the *vapBC* TA loci found in *S. solfataricus* [36]. Some TA loci were constitutively expressed at high levels (e.g. *vapBC22*), but thermal stress triggered even higher transcription levels (Figure 2). Other TA loci were significantly up-regulated by thermal stress, such as *vapBC6* and *vapBC8* of *S. solfataricus*. Using *S. solfataricus* strain PBL2025, genetic insertions were made to disrupt the function of individual TA genes [41]. When toxin *vapC22* was disrupted, no obvious phenotype was noted, although approx. 100 ORFS were differentially transcribed 2-fold or more (C.R. Cooper, S. Tachdjain, A.J. Daugherty, P.H. Blum and R.M. Kelly, unpublished work). However, disruption of *vapB6* (and consequently *vapC6*) rendered the organism susceptible to thermal stress (C.R. Cooper, S. Tachdjain, A.J. Daugherty, P.H. Blum and R.M. Kelly, unpublished work). Efforts are now underway to seek to determine the set of essential TA loci required by *S. solfataricus* to survive thermal stress.

Concluding remarks

Because many TA loci are still annotated as ‘hypothetical proteins’, they are often overlooked as important elements in microbial genomes. Transcriptional data from *S. solfataricus* heat-shock experiments suggested that, although chromosomally encoded TA loci may not play a significant role in mesophilic prokaryotes such as *E. coli*, they are potentially significant stress-response modules in thermophilic archaea. One hypothesis yet to be tested is that TA loci are key components in archaeal RNA management systems. The significance of the large complement of TA loci in thermophilic archaea (as noted, there are 26 *vapBC* loci in *S. solfataricus* alone) remains a mystery. Each locus may play a specific role under normal or stress conditions. Further work is needed to define the targets of specific VapCs in *S. solfataricus* as this may lead to a clearer picture of TA loci in archaeal biology.

Acknowledgments

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Abbreviations used

HSP	heat-shock protein
ORF	open reading frame
PIN	Pil-T N-terminus
PSK	post-segregational killing
sHSP	small heat-shock protein
TA	toxin–antitoxin
vap	virulence-associated protein

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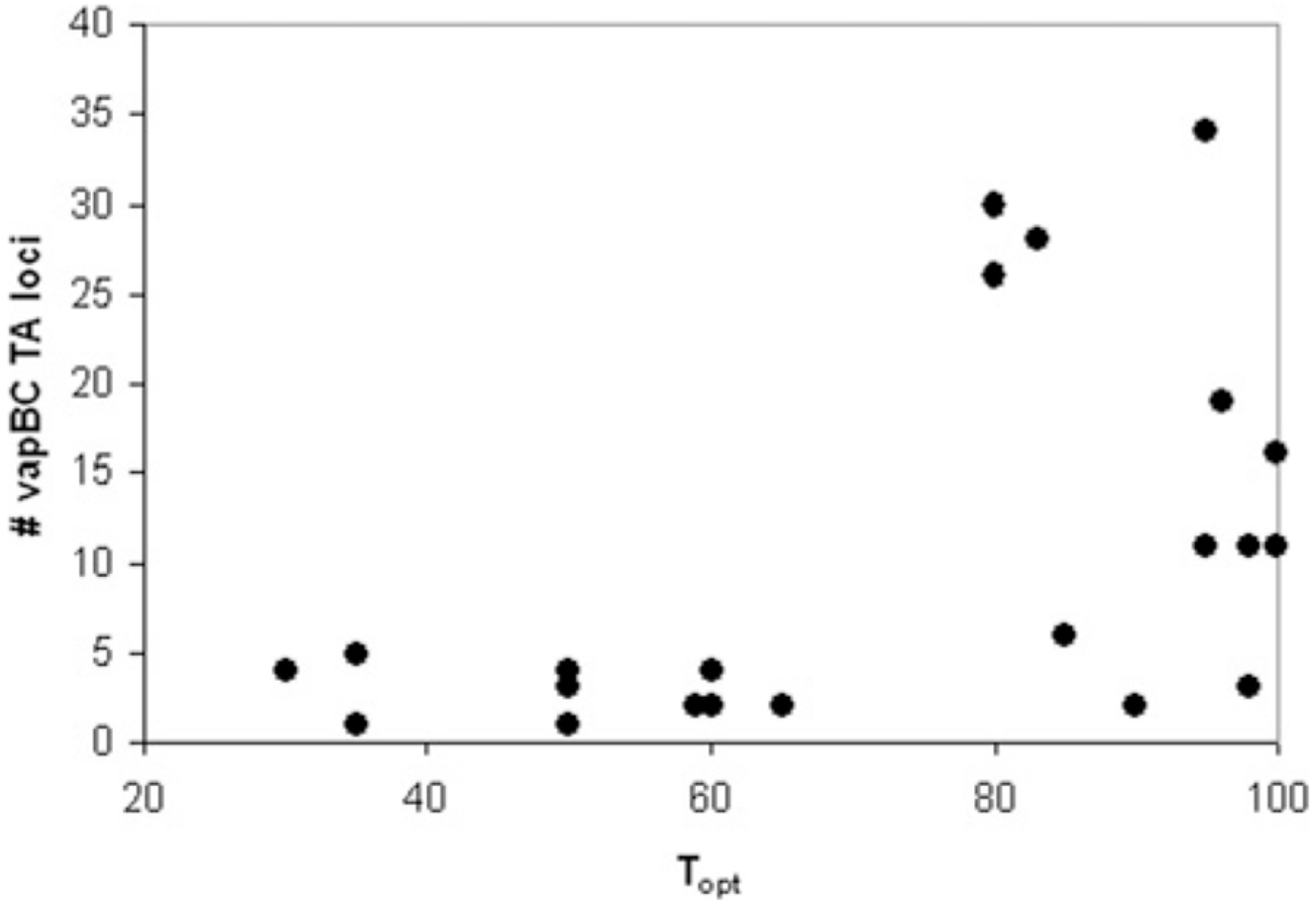

Figure 1. The number of chromosomally encoded *vapBC* TA loci increases with optimal growth temperature for genome-sequenced archaea

T_{opt} data were obtained from The Prokaryotic Growth Temperature Database (http://pgtdb.csie.ncu.edu.tw/).

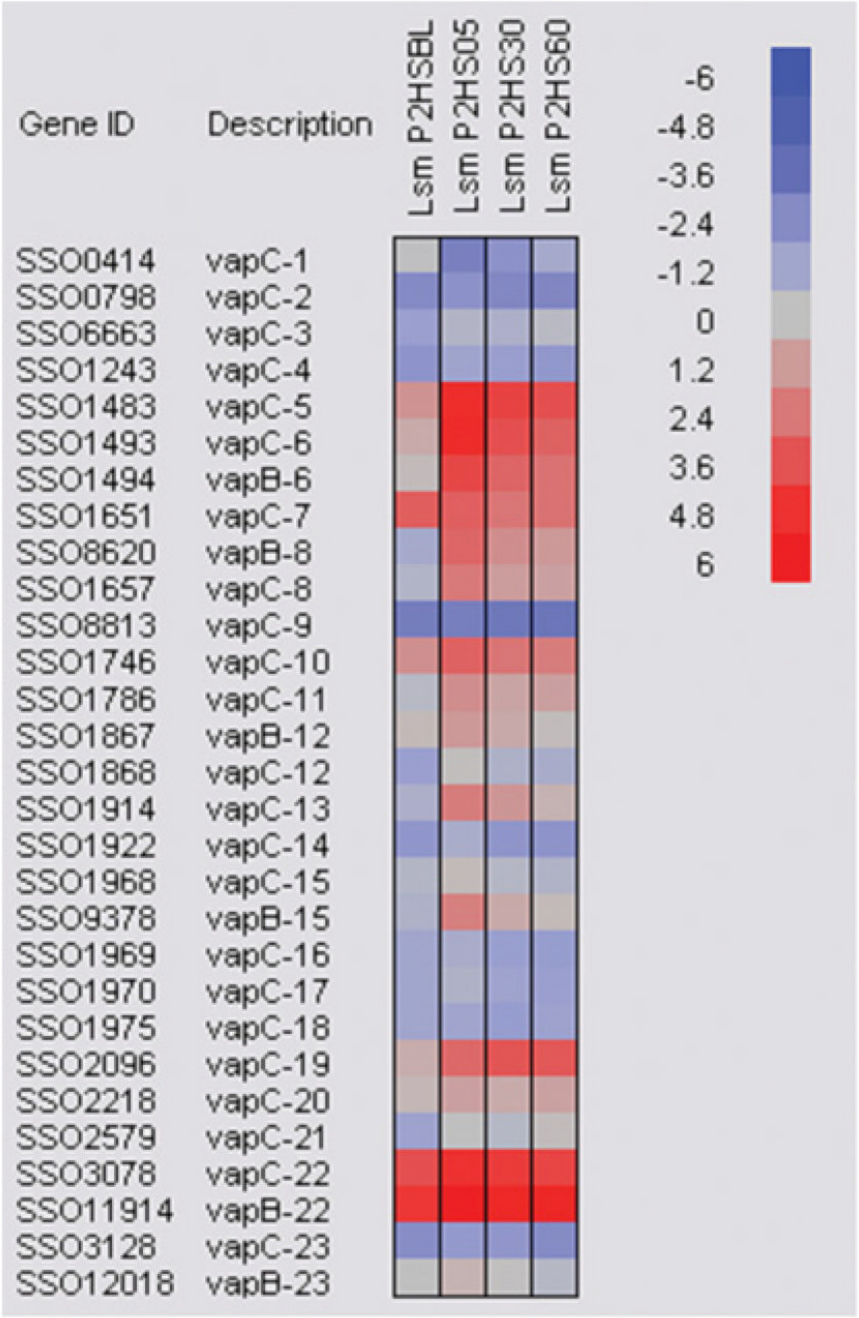

Figure 2. *Sulfolobus solfataricus* P2 *vapBC* TA loci transcriptome before and after heat shock (temperature shift from 80 to 90°C)

Red indicates up-regulation and blue represents down-regulation, relative to the genome-wide average transcription level. Missing *vapB*s were not annotated at the time the DNA microarray used here was fabricated.