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Commentary

Commentary on "Type II Toxin-antitoxin Systems Have a Peculiar Localization in *Escherichia Coli* Cells"

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Introduction

Bacterial Toxin-Antitoxin (TA) modules were extensively studied [1-3]. They are located on extra chromosomal genetic elements as well as on bacterial chromosomes. They carry two genes. One encoding for the toxin, and the other for the antitoxin. The toxin is a stable protein, while the antitoxin is either RNA or a labile protein. They are divided into six groups [3,4], among them group II is the most studied. In-group II both toxin and antitoxin are proteins [5]. To this group belongs the *E. coli* TA system *mazEF* which is the first bacterial chromosomal TA discovered, and is since extensively investigated [6-9]. The toxin MazF is a stable protein while the antitoxin MazE is degraded by ClpX protease [6]. *mazEF* is triggered by all kind of stressful conditions like rifampicin, chloramphenicol, spectinomycin, high temperature (50°C) [7,10], DNA damage (UV irradiation, nalidixic acid, mitomycin C. thymine starvation) and oxidative stress (H_2O_2) [7,10-12]. Each of these stressful conditions has been shown to lead to *E. coli mazEF*-dependent cell death, which occurs only at logarithmic stage of growth in minimal medium. In addition, *E. coli mazEF*-mediated cell death is a population phenomenon requiring a unique *E. coli* quorum sensing (QS) factor, the Extracellular Death Factor (EDF) [13,14]. By structural analysis, *E. coli* EDF (*Ec*EDF) was identified as the linear penta-peptide Asn-Asn-Trp-Asn-Asn (NNWNN) [13]. *Ec*EDF amplifies both the endoribonucleolytic activities of *E. coli* MazF and overcomes the inhibitory activity of the *E. coli* antitoxin MazE over the toxin MazF [15]. *E. coli* MazF is a sequence-specific endoribonuclease that preferentially cleaves single-stranded mRNAs at ACA sites [16], and thereby generate a special translation machinery designated STM (Stress Translation Machinery [17]. This occurs by the following process: a) MazF creates "stress mRNAs" by cleaving at ACA sites immediately upstream to [17], or further upstream [18] from the AUG-start codons of specific mRNAs; b) by targeting an ACA site in the 16S rRNA within the 30S ribosomal subunit at the decoding center, MazF removes 43 nucleotides from the 3'-terminus, including the anti-Shine Dalgarno region [17].

Here we investigated the intracellular localization of the protein products of the following type II TA modules: *mazEF*, *chpBIK*, *mqsRA and rnlAB* [19]. We followed the localization of these proteins by fusing them with the fluorescent protein mCherry. Then, we used fluorescent microscopy and image analysis software in order to obtain the protein distribution data.

Our results revealed that with the exception of the *chpBIK* TA module, the localization of each toxin-antitoxin complex differ from the localization of the toxin by itself. Thus, our results clearly show that the antitoxin shifts the localization of its respective toxin towards the center of the cell. Probably such a shift contributes to a reduction of cellular toxicity.

Results and Discussion

In contrast to our expectation each of the studied TA modules had its own pattern of localization for the toxin, the antitoxin, and the toxin-antitoxin complex (Figure 1). With the exception of the *chpBIK* TA module, for each TA system, the localization of the toxin-antitoxin complex differs the localization of the toxin by itself. The presence of the antitoxin shifts the toxin from the pole towards the center of the cells. Therefore, we suggest that in contrast to the generally considered view, the antagonistic effect of antitoxins on their cognate toxins is not only based on their direct structural interactions. But it is also

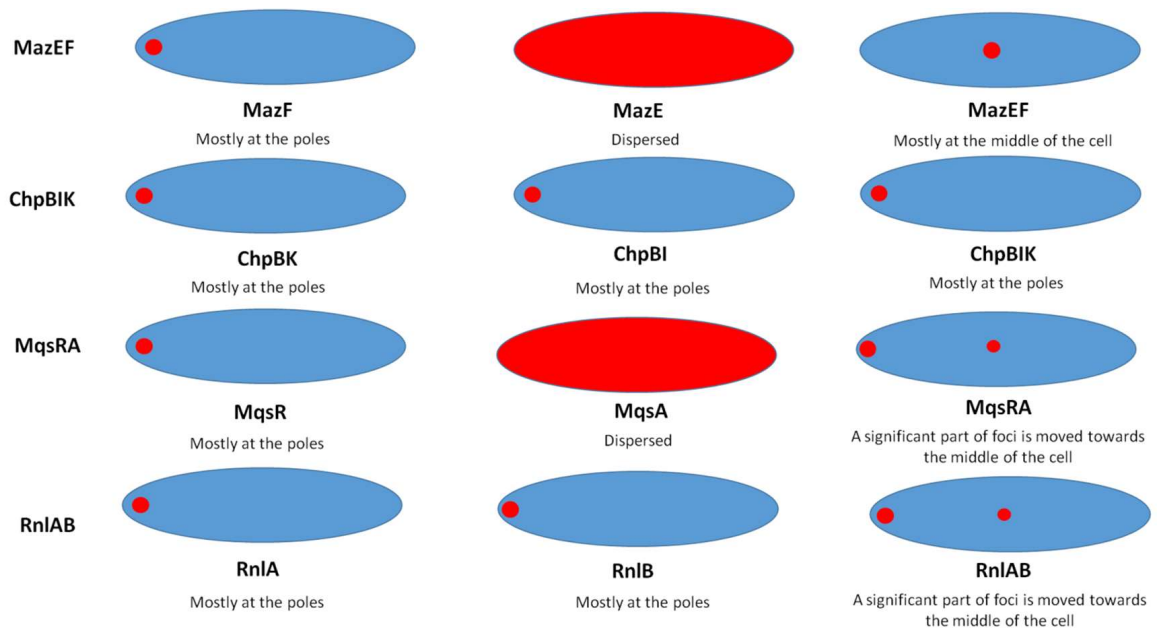

Figure 1: A schematic representation of the intracellular localization of the protein products of some type II TA systems in *E. coli.*

The representation was taken from the fluorescent microscopy images of the protein-mCherry fusions [19]. Fluorescence is represented by red color. Larger red circles represent polar localization (MazF and ChpBK), and smaller red circles represent the localization at the middle of the cells (MqsRA, RnlAB). Dispersed localization (MazE, MqsA) is represented as a full colored red bacterial cell.

caused by changing the intracellular localization of the toxin. The reasons for the migration of the toxins toward the poles, and their shifts toward the center of the cells in the presence of the antitoxins, are subjects for future experiments.

Further studies should identify the *E. coli* component(s) that provide the peculiar location pattern of group II TA modules. Is it related to *E. coli* proteins that were previously identified as being critical for cell division [20-22]? Or to those that have other functions and were identified to have a polar localization [23-24]? Alternatively, is the polar localization connected to the yet unknown location of the STM or EDF systems that are unique at least in the case of MazF?

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