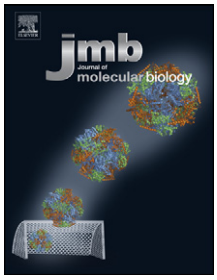

The RavA-ViaA Chaperone-Like System Interacts with and Modulates the Activity of the Fumarate Reductase Respiratory Complex

Keith S. Wong [1,†], Vaibhav Bhandari [1,†], Sarath Chandra Janga [2] and Walid A. Houry [1,3]

*1 - **Department of Biochemistry,** University of Toronto, Toronto, Ontario, M5G 1M1, Canada*
*2 - **Department of Biohealth Informatics,** School of Informatics and Computing, Indiana University Purdue University, Indianapolis, IN 46202, USA*
*3 - **Department of Chemistry,** University of Toronto, Toronto, Ontario, M5S 3H6, Canada*

***Correspondence to Walid A. Houry:** Department of Biochemistry, Faculty of Medicine, University of Toronto, MaRS Centre, West Tower, 661 University Avenue, Suite 1600, Toronto, Ontario M5G 1M1, Canada. walid.houry@utoronto.ca*
http://dx.doi.org/10.1016/j.jmb.2016.12.008
Edited by Charalampo Kalodimos

Abstract

*R*egulatory *A*TPase *v*ariant *A* (RavA) is a MoxR AAA+ protein that functions together with a partner protein that we termed *V*WA *i*nteracting with *A*AA+ *A*TPase (ViaA) containing a von Willebrand Factor A domain. However, the functional role of RavA-ViaA in the cell is not yet well established. Here, we show that RavA-ViaA are functionally associated with anaerobic respiration in *Escherichia coli* through interactions with the fumarate reductase (Frd) electron transport complex. Expression analysis of *ravA* and *viaA* genes showed that both proteins are co-expressed with multiple anaerobic respiratory genes, many of which are regulated by the anaerobic transcriptional regulator Fnr. Consistently, the expression of both *ravA* and *viaA* was found to be dependent on Fnr in cells grown under oxygen-limiting condition. ViaA was found to physically interact with FrdA, the flavin-containing subunit of the Frd complex. Both RavA and the Fe–S-containing subunit of the Frd complex, FrdB, regulate this interaction. Importantly, Frd activity was observed to increase in the absence of RavA and ViaA. This indicates that RavA and ViaA modulate the activity of the Frd complex, signifying a potential regulatory chaperone-like function for RavA-ViaA during bacterial anaerobic respiration with fumarate as the terminal electron acceptor.

Introduction

The MoxR family of AAA+ ATPases is relatively unknown, although it is diverse and widespread among bacteria and archaea [1]. The experimental evidence gathered on various MoxR proteins suggests that they have regulatory and chaperone-like roles in the maturation of protein complexes participating in a variety of biological processes including metabolism, cell morphology and development, tolerance against various types of stress, and pathogenesis [1–4]. A characteristic of the MoxR AAA+ ATPases is that their genes co-occur in close proximity with one or more genes whose proteins contain the von Willebrand factor A (VWA) domain [1]. The VWA domain features the conserved MIDAS (*m*etal *i*on-*d*ependent *a*dhesion *s*ite) motif, which binds a single divalent cation, usually Mg^{2+}, and mediates protein–protein interactions [5].

The functional characterization of the MoxR protein *r*egulatory *A*TPase *v*ariant *A* (RavA) and its corresponding VWA protein *V*WA *i*nteracting with *A*AA+ *A*TPase (ViaA) in *Escherichia coli* is an ongoing effort in our laboratory. RavA belongs to the eponymous RavA subfamily of the MoxR family [1]. The *ravA* and *viaA* genes are organized in a pattern that is typical of this subfamily with *ravA* positioned immediately upstream of *viaA* and with both genes forming a single operon [6]. In aerobically grown cells, the *ravAviaA* operon is induced by the stationary phase sigma factor, σ^S [6]. RavA has been characterized

extensively by our group both biochemically and biophysically. It forms a hexameric complex [6,7], which is typical for most AAA+ ATPases [8]. *In vitro*, the ATPase activity of RavA is optimal at neutral pH and 37 °C, which is enhanced in the presence of ViaA [6]. In stationary phase cells, RavA was found to mainly localize to the cytoplasm, while ViaA was found to be localized to both the cytoplasm and the inner membrane [9].

Although little is known about their cellular function, several interaction partners for RavA have been identified that suggest its involvement in potential regulatory roles in different biological processes. For example, RavA associates with and modulates the activity of the inducible lysine decarboxylase (LdcI) [6,7,10,11], a major acid stress response protein in *E. coli* [10,12]. The alarmone, ppGpp, was found to bind and inhibit the activity of LdcI, and the interaction of RavA with LdcI prevented this binding of ppGpp to LdcI [7]. This supports a potential role of RavA, and possibly ViaA, in bacterial acid stress response [7]. In addition, RavA and ViaA were functionally linked to bacterial respiration when they were shown to sensitize the cell to aminoglycosides [9,13]. The identification of null mutations that suppressed this phenotype, and subsequent immunoprecipitation experiments, revealed that RavA and ViaA interact with specific subunits of the NADH:ubiquinone oxidoreductase I (Nuo complex) [9]. The Nuo complex, commonly known as Complex I, is a major player in the aerobic respiration of *E. coli* [14,15]. It is also important in anaerobic respiration utilizing fumarate and dimethylsulfoxide [16].

High-throughput studies have revealed functional links between RavA-ViaA and several pathways that are directly or indirectly related to bacterial respiration. These include iron–sulfur (Fe–S) cluster biosynthesis, iron transport, and anaerobic electron transport [9,17]. In this study, we present evidence that supports a regulatory role of the RavA-ViaA proteins in the activity of the anaerobic respiratory complex fumarate reductase (Frd). The *E. coli* Frd complex catalyzes the final step of anaerobic respiration when fumarate acts as the terminal electron acceptor [18]. The complex is formed by four subunits (FrdABCD) [19,20] with FrdC and FrdD being the membrane-spanning subunits, while the flavoprotein FrdA and the iron–sulfur cluster-containing protein FrdB comprise the soluble part of the complex. During anaerobic respiration, menaquinol (MQH2) in the membrane donates electrons to the Frd complex [21]. The membrane-spanning FrdCD subcomplex anchors the FrdAB components to the membrane and, along with FrdB, provides binding sites for the quinones. The electrons then traverse through the three iron–sulfur clusters present in FrdB to the flavin adenine dinucleotide (FAD) cofactor in the FrdA active site where they are used to reduce fumarate to succinate [20–23]. Here, we find ViaA to interact with free FrdA through its C-terminal VWA domain and with RavA through its N-terminal α-helical rich domain. Importantly, the interaction of RavA-ViaA with FrdA results in a decrease in Frd activity. A model of the effect of RavA-ViaA on the maturation of the Frd complex is proposed.

Results

ravA and *viaA* display similar co-expression profiles as those of the Fnr-inducible genes

We had earlier demonstrated that RavA-ViaA interact with LdcI and the Nuo complex [2,6,9]. However, given that we postulated that RavA-ViaA might have chaperone-like activity [1,2], further studies were carried out to identify new interacting partners for this system. Initially, co-expression profiling was performed to identify genes that co-express with both *ravA* and *viaA*. This approach is based on the principle that genes are organized in a network of distinct, functional modules or hubs with highly coordinated expression patterns that correspond to specific biological processes [24–27]. Thus, genes that are functionally associated have a higher likelihood of sharing common transcriptional regulatory elements and of displaying similar expression profiles in response to the same physiological signals or external environmental stimuli.

The co-expression profiles for *ravA* and *viaA* genes were constructed by data mining a public collection of 445 *E. coli* microarray datasets collected across multiple experimental conditions, and then, genes that displayed highly similar co-expression patterns with *ravA* and *viaA* were identified. Our analysis yielded a total of 62 genes that co-express with *ravA* and 56 genes that co-express with *viaA* (Fig. 1a and Table 1). Of these, 32 genes co-express with both *ravA* and *viaA*. Given that *ravA* and *viaA* are in the same operon [1,6], genes that are co-expressed with both *ravA* and *viaA* were considered as the most reliable candidates for functional association and were examined further.

One important trend uncovered in our analysis is that many of the genes that co-express with both *ravA* and *viaA* are involved in anaerobic respiration. These include *frdA*, *frdB*, and *frdC*, which encode three of the four subunits of the Frd complex (FrdABCD); *nirB* and *nirD*, which encode the large and small subunits, respectively, of the nitrite reductase complex ($NirDB_2$); *hybO*, which encodes the small subunit of hydrogenase 2 (HybABOC); and *nrfA*, which is the structural gene for cytochrome c_{552} and a component of the formate-dependent nitrite reductase complex (NrfDCBA).

A second group of genes falls under protein maturation and modification, all of which—*hypA*, *hypB*,