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A way to identify archaellins in *Halobacterium salinarum* archaella by FLAG-tagging

Research Article

Sergei N. Beznosov[1,*], Michael G. Pyatibratov[1], Pavan S. Veluri[2], Sagar Mitra[2], Oleg V. Fedorov[1]

[1]*Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Russia*

[2]*Department of Energy Science and Engineering, Indian Institute of Technology, 400076 Mumbai, India*

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Abstract: In the current study, haloarchaea *Halobacterium salinarum* cells were transformed individually with each of the modified archaellin genes (*flaA1*, *flaA2* and *flaB2*) containing an oligonucleotide insert encoding the FLAG peptide (DYKDDDDK). The insertion site was selected to expose the FLAG peptide on the archaella filament surface. Three types of transformed cells synthesizing archaella, containing A1, A2, or B2 archaellin modified with FLAG peptide were obtained. Electron microscopy of archaella has demonstrated that in each case the FLAG peptide is available for the specific antibody binding. It was shown for the first time that the B2 archaellin, like archaellins A1 and A2, is found along the whole filament length.

Keywords: *Haloarchaea • Archaeal flagella • Flagellin • Immuno-electron microscopy*

1. Introduction

Archaeal flagella (archaella), despite their morphological similarity to bacterial flagella, are fundamentally different structures [1]. Typically, the archaella are thinner (10-13 nm) compared to bacterial flagella (20 nm) [2]. Archaeal flagellins (archaellins) have no homology to bacterial flagellins, but at the same time, they are homologous in N-termini to type IV bacterial pilins [3] and are processed in a similar manner, with the removal of the leader peptide before incorporation into supramolecular structure [4]. Archaellins in the archaellum are often glycosylated [3]. Among the Archaea the variety in the number of archaellins in the archaellum is significantly higher (1 to 8) [5] than that of flagellins in Bacteria. Recent data indicate that the Archaea may differ in the mechanism of forming the spiral shape of the archaellar filament [5,6]. Sometimes a mechanism similar to that in eubacteria occurs [5]; in this case a single protein subunit forming the flagellar filaments possesses the ability to adopt either one of the two conformations, forming two types of protofilaments, which differ in length and generate the filament curling upon association [7]. Other mechanisms require multicomponent archaella composition [8-10] or the presence of multiple archaellin forms having different modifications [5]. Most studies on the role of multiple archaellins used methods of inactivation and deletion of genes [3]. Jarrell and co-workers have identified and localized minor archaellins FlaA1 and FlaB3 in the *Methanococcus voltae* filament, using antibodies specific to the variable regions of archaellins, and showed that FlaA1 is distributed over the entire length of the filament, while FlaB3 forms a short curved hook proximal to the cell [11].

The archaella filament of haloarchaea *Halobacterium salinarum* consists of five archaellins - A1, A2, B1, B2 and B3 (FlgA1-FlgB3), which are encoded by genes located in the *flgA* and *flgB* operons [12,13]. Archaellins A1 and A2 are major filament components, while B1, B2 and B3 are synthesized in much smaller quantities [8]. Previously, by deletion of archaellin genes, it was shown that the helical filaments may be formed only from

* *E-mail: supra@vega.protres.ru*