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Structural biology of *Helicobacter pylori* type IV secretion system

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Background

Helicobacter pylori chronically infects the gastric mucosa of millions of people annually worldwide: it has been estimated that over 50% of the world population carries this infection. *H. pylori* has been associated with the development of several diseases, like chronic gastritis, gastric and duodenal ulcer, gastric adenocarcinoma and mucosa-associated lymphoma [1-3].

The complete genome sequence of two different isolates of *H. pylori* (J99 and 26995) is known. The strains that contain a 37 kb foreign DNA region, called *cag* pathogenicity island (*cag*-PAI), cause the most severe form of virulence [4].

The *cag*-PAI encodes for a functional type IV secretion apparatus homologous to the VirB/D4 Type IV Secretion System (T4SS) of the plant pathogen *Agrobacterium tumefaciens* and other Gram-negative bacteria [5]. T4SSs are involved in conjugal DNA transfer, in the DNA delivery to (or uptake from) the environment, for instance the release of oncogenic DNA into infected plant cells by *A. tumefaciens*, or in the translocation of effector proteins [6,7].

The T4SS encoded by the *cag*-PAI of *H. pylori* is responsible for the translocation into the host cell of the protein CagA, a major antigenic virulence factor encoded within the *cag*-PAI. Once secreted into the gastric epithelial cells, CagA induces cellular modifications, such as elongation and spreading of host cells [8].

The aim of this structural genomic project is to determine the three-dimensional structure of most of the proteins encoded by the *cag*-PAI, a task that will allow to elucidate the function and the organization of the entire T4SS of such a relevant pathogenic bacterium [9].

Results

Protein production for structural studies presents one of the most difficult and challenging tasks for heterologous expression in *E. coli*. Generally, the protein must be native, active, soluble, highly pure, and concentrated. We have identified protein insolubility/aggregation as the major bottlenecks in the process towards the determination of protein structures by X-ray diffraction. Each protein often needs separate handling and analysis to determine tag choice, growth and buffer conditions for optimal solubility. To speed up recombinant protein production, we have adopted a strategy of parallel expression of a protein from a variety of vectors containing different tags and/or fusion partners, and a variety of *E. coli* host strains.

To this point in time, we have cloned, expressed, and purified several proteins of the *cag* pathogenicity island of *H. pylori*. They all have been expressed in *E. coli*. We have already determined the structure of CagZ [10] and CagS, using the Se-Met method. We have also obtained crystals of an other protein, along with crystallization tests on other *cag* proteins.

Perspectives

We believe these studies will also furnish valuable information for vaccine production and provide insights into the mechanism of *H. pylori* pathogenesis.

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