

Complexome dynamics during type III protein secretion by Enteropathogenic Escherichia coli

Michalis Aivaliotis¹, Athina Portaliou^{1,2}, Vassilia Balabanidou^{1,2}, Spyridoula Karamanou¹,
Anastassios Economou^{1,2}

1. IMBB-FORTH, Heraklion, Crete, Greece
2. Dpt of Biology, U. Crete, Heraklion, Crete, Greece

Introduction

Enteropathogenic Escherichia coli (EPEC), a bacterial pathogen, causes diarrheal disease [1]. Type III secretion system (T3SS) encoded by a pathogenicity island called the locus of enterocyte effacement (LEE) is the central element of its pathogenesis [2,3]. T3SS injects LEE and non-LEE-encoded effector proteins into the host cell, where these effectors modulate host signaling pathways and immune responses [3,4]. Although a plethora of studies have expanded our knowledge on the structure and function of T3SS and its secreted effectors, the precise mechanisms of type III protein secretion, translocation and their co-ordination remain poorly understood. To better understand these mechanisms, a comprehensive analysis of the protein complexes during Type III protein secretion is required. Here we present a complexome analysis of EPEC.

Materials and Methods

In a global approach, cytosolic protein complexes were isolated and fractionated by Native polyacrylamide gel electrophoresis, while in a targeted approach, selected His-tagged T3SS-related proteins were used as baits for the selective isolation of T3SS-related protein complexes which were further separated by Native polyacrylamide gel electrophoresis. Protein subunits of the complexes were analyzed by “bottom-up” proteomics using an Easy-nLC coupled via a nanoESI source to a LTQ-Orbitrap. Peptides were injected onto an in-house packed 10cm, C18, fused silica emitter column, and eluted into the LTQ-Orbitrap using a 175 min linear gradient of increasing ACN concentration. Protein identification was performed by Proteome Discoverer, using both Mascot and Sequest. Scaffold was used for statistical evaluation of the data.

Results

The combination of a global and a targeted approach, with optimized Native-PAGE and high accuracy mass spectrometry yielded the reliable identification of more than 1300 unique cytosolic proteins from EPEC cells. This corresponds to ~28% of its total theoretical proteome and ~50% of its predicted cytosolic proteome. The identified proteins represent a wide range of cellular processes as revealed from their GO annotations which is required for a reliable and comprehensive subsequent complexome and network analysis. Global complexome analysis determined more than 150 putative cytosolic protein complexes in EPEC. These include many complexes previously reported in laboratory strains of E.coli, providing validation for our approach. Targeted complexome analysis focused on the 54 predicted T3SS-related proteins. Several T3SS pre-secretion protein complexes and interactions were thus identified in the EPEC cytosol against a background of the house-keeping complexome. More than 15 cytosolic key proteins of EPEC pathogenesis such as EspA, EspB, CesAB, Tir, CesT and Map were identified as subunits of these protein complexes. In an effort to verify these protein complexes and elucidate their precise function during T3S protein secretion, immuno-detection and knock-out mutants were used.

References

- [1] Pallen, M. J., and B. W. Wren, *Nature*, 449:835–842 (2007)
- [2] Iguchi A. et al., *J. Bacteriology*, 191, 347–354 (2009)
- [3] Dean P. & Kenny B., *Current Opinion in Microbiology*, 12:101–109 (2009)
- [4] Garmendia, J., G. Frankel, and V. F. Crepin, *Infect. Immun.*, 73:2573–2585 (2005)

Novel Aspects

This is the first comprehensive complexome analysis of the human pathogen EPEC targeting the cytosolic T3SS-related complexome.