Dynamic Complexome and Secretome Analysis for the study of Type III Protein Translocation and Secretion Mechanisms

Michalis Aivaliotis[1], Athina Portaliou[1,2], Vassilia Balabanidou[1,2], Nikolaos Kontourakis[1], Dimitra Chronaki[1,2],Georgia Orfanoudaki[1,2], Spyridoula Karamanou[1], Anastassios Economou[1,2]

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

Introduction

The type III secretion system (T3SS) is a specialized bacterial protein secretory pathway that plays an essential role in the pathogenesis of Gram-negative bacteria (e.g. Enteropathogenic *E. coli*, EPEC) [1]. It is encoded by the Locus of Enterocyte Effacement (LEE), and injects effector proteins into the host cell, modulating key cellular processes [2]. The precise mechanisms of T3SS remain poorly understood.

Methods

Cytosolic protein complexes were isolated from wt and selected deletion mutant and fractionated by Native polyacrylamide gel electrophoresis (N-PAGE) and size-exclusion chromatography (SEC). In a targeted approach, His-tagged T3SS-related proteins were used for the isolation of protein complexes which were fractionated by N-PAGE and SEC. The secretome was collected and concentrated by acid-mediated precipitation. Complexome and secretome were analyzed by "bottom-up" proteomics. Protein identification, validation and relative quantitation was performed by Proteome Discoverer, Scaffold, and iBAQ [3], respectively.

Results

More than 1500 proteins from a wide range of cellular processes were identified, that corresponds to >80% of the expressed cytosolic proteome. As a proof of principle, ~150 known and novel protein complexes were determined. 38 predicted as non-transmembrane T3SS-related proteins, were identified as components of several T3SS protein complexes and interactions. Immuno-detection, pull downs and deletion mutants were used for the verification and the elucidation of the precise function of these complexes during T3 protein secretion.

Conclusions

The combination/optimization of global and targeted approaches for the characterization of protein complexes shed light on the dynamics of T3SS-related cytosolic complexome. Known and novel complexes and interactions were determined. Further in depth study of the identified complexes will provide us with new insights on the poorly understood T3SS mechanisms.

References

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