Temporal profiling of newly translated proteome in Mycobacterium tuberculosis Infected macrophage cells

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Macrophase proteome, in contrast to its complimentary genome, seems boundless in response to Mycobacterium tuberculosis (MtB) infection. Consequently, a protein could be present in different forms and/or amounts at different times. In this study, an effort was made to capture this panoramic view by specifically targeting newly translated host proteins over time, in response to MtB infection, by blending two discrete state-of-the-art approaches – click chemistry and SILAC labeling. Such a unified approach allows cells to be studied irrespective of complexity of the background proteins. PMA differentiated THP1 cells were infected with H37Ra (avirulent) and H37Rv (virulent) strains of MtB. The variety of newly synthesized proteins was captured by azidohomoalanine incorporation over twelve 4 hour windows from 0 - 50 hours of infection. Cell lysates from light (K0R0), medium (K6R6) and heavy (K8R10) SILAC labeled cells were pooled, for respective time windows, and enriched for newly translated proteins using Click-iT Protein Enrichment kit. Samples were subsequently digested and analyzed by reverse-phase high-pressure liquid chromatography electrospray ionization tandem mass spectrometry on ABSCIEX 5600 Triple-TOF platform. A total of 1180 newly translated host proteins were identified in response to MtB infection. Of these, 810 macrophage proteins showed differential expression. These included proteins involved in metabolism, apoptosis and immune response, as well as other cellular processes. Since almost 70% of host proteome is observed to be perturbed, we conclude that the host is constantly adapting to its changing environments in the presence of a pathogen. The experimental approach employed in this study can contribute to an increased understanding of the dynamic host-pathogen interactions and consequential cellular communication and responsiveness over time.

In Situ Cell-Surface Proteomics: Method Development and Applications in Neurobiology

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Cell-surface molecules are direct executors for inter-cellular communications and thus regulate almost every aspect of the development and physiology of multicellular systems. We developed a method for quantitatively profiling cell-surface proteomes in native tissues with cell-type and spatio-temporal specificities. Applying this method to developing brains uncovers many novel molecules controlling neural circuit assembly, as well as their operating mechanisms. Here, I will share our discoveries made by in situ cell-surface proteomic profiling: 1) how unconventional wiring molecules control dendrite targeting; 2) how a lineage-defining transcription factor specifies a combinatorial cell-surface code for olfactory circuit assembly; and 3) how an endocytosis-associated cell-surface protein regulates Purkinje cell dendrite morphogenesis.

100360, https://doi.org/10.1016/j.mcpro.2022.100381