Encl. 19D

Details of earlier grant received from US : (Completion Report)

Title : High-throughput substrate profiling for *Mycobacterium tuberculosis* Protein Kinase K using Tuberculosis (TB)-Nucleic Acid Programmable Protein array (NAPPA) technology

Role: Co-Principal Investigator

Funding Agency : Arizona Community Foundation

Duration - 1/11/2011-30/11/2012

Grant size – $ 20,000 USD

Objectives:

The Objectives of this study were to develop and use the high throughput Nucleic Acid Programmable Array (NAPPA) platform for identifying substrates for the *Mycobacterium tuberculosis* Serine/Threonine Protein kinase K (PknK) and to identify *M. tuberculosis* proteins that are potential targets for development of new antibiotics.

Achievements:

NAPPA is a novel protein microarray technology, developed by Dr. Joshua LaBaer which consists of arrays of the genes encoding proteins from a specific organism (in this case, *M. tuberculosis*). Each gene is fused to the coding sequence for a tag protein, glutathione *S*-transferase (GST) and "printed" on a microarray slide. The genes are then transcribed and translated by a cell-free system and the proteins remain in place due to the GST tags.

High throughput protein-protein interaction in NAPPA

DNA fragments encoding wild-type *M. tuberculosis pknK* and phosphorylation-defective *pknK* (generated by mutating the *pknK* gene at a specific site that resulted in the substitution of a critical amino acid in the phosphorylation site of the protein; this is designated as *pknK* K55M) genes were cloned into a plasmid vector designated pJFT7_N_Halo. The wild-type and mutant ***pknK*** genes were expressed as fusion proteins with the Halo tag and were used as the "query" proteins with the NAPPA protein arrays in protein-protein interaction assays.

The pJFT7_N_Halo vectors with the wild-type and mutant *pknK* genes were successfully generated and the protein expression was confirmed by Western blot. NAPPA arrays containing 2000 *M. tuberculosis* genes were screened for protein-protein interactions with PknK and PknK K55M proteins, with the pJFT7_N_Halo empty vector (expresses only the Halo tag) or with the Human In Vitro Transcription-Translation (IVTT) reaction mix without any DNA (Negative control). More than 50 genes were identified as potential candidates interacting with PknK proteins. The kinase activity of PknK seems to be relevant for a subset of the interactions detected on the array (14 out of 50), as no signal was detected on the arrays probed with the phosphorylation-defective protein (PknK K55M). The screening was performed twice and 5 genes (**MT3037, Rv0889c, Rv1037c, Rv1536,**

Rv2347c) were identified as hits in both experiments (Fig1). More experiments will be performed to substantiate the assay reproducibility and the number of hits detected in multiple assays and results will be validated by independent assays (immunoprecipitation or complementary fluorescent assays).

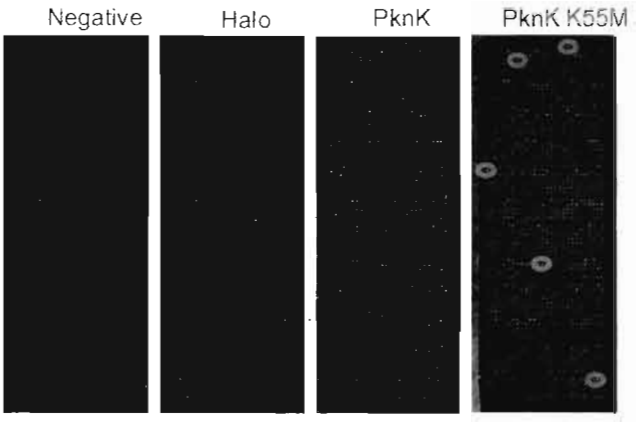

Fig. 1. Hybridization of PknK and PknK K55M proteins on the NAPPA chips.

True Copy
Vandana Malhotra
28/9/2020