

Role-Mining the Moonlighting GAPDH Proteins in *Cucumber Mosaic Virus* Resistance in Plants

Bipasha Bhattacharjee*

Plant Virology Laboratory, Biotechnology Division, CSIR-Institute of Himalayan
Bioresource Technology (CSIR-IHBT), Palampur, H.P., India
Email: bipashabhattacharjee92@gmail.com

“Research is to see what everybody else has seen, and to think what nobody else has thought”, this is a great description of research by the Nobel *laureate*, Albert Szent-Györgyi. I shall begin with the description of my initial foray into science which had been nothing short of one of the most innocent venture, enrapturement and excitement. Inescapable reasoning and logic in science being steadfast and dependable, explained all the questions that kept cropping up in my curious young mind. It was easy and satisfying and I felt I needed to comprehend and learn more. Science has evolved from an interesting textbook topic to one of the most intriguing and intense passion, woven into my life. Science, and more particularly research according to me are fluid, dynamic and challenging every day and everything that surrounds us.

Plants and viruses have always fascinated me. Hence, I chose to pursue plant virology as the topic of my doctoral study. It is one of the most interesting study topics as focus on this aspect of biotic stress is limited in terms of funding as well as understanding. Viruses work in such pluri-directional conduits that many a times to pinpoint its activity becomes tedious. This is why we try to perform immaculate research in our laboratory at CSIR-IHBT, Palampur to characterise elements which could take part in controlling the spread of viruses in endemic crops which could lead to viro-tolerant varieties of commercially important plants.

Moonlighting proteins are *coup de foudre* for researchers of a wide array of fields, as they participate in a humongous variety of functionalities across multitudes of mechanisms across the

* Ms. Bipasha Bhattacharjee, Ph.D. Scholar from CSIR-Institute of Himalayan Bioresource Technology, Palampur, is pursuing her research on “Ascertaining the Role of Gapdh in Viral Pathogenesis.” Her popular science story entitled “Role-Mining the Moonlighting Gapdh Proteins in Cucumber Mosaic Virus Resistance in Plants” has been selected for AWSAR Award.

biological spectrum. Targeting these proteins constantly opens new opportunities for understanding their interactions with other proteins which can affect the structure of a particular mechanistic pattern. What makes their study even more unique is the fact that they function in a very intriguing manner their different functions are disparate and autonomous, opening up a plethora of possibilities in mining their roles in metabolism, developmental and defence processes. Such proteins have been proposed to vary in their functionalities according to their cell type, sub-cellular localisation, state and mode of action, difference in cellular component concentrates, presence or absence of ligands and cofactors and post-translational modifications. Metabolite binding to these proteins, protein-protein interactions or multi-protein complex formation can infer greatly upon their function, while these strategies not being mutually exclusive and can many a times take place in cohesion. Different annotations are conferred upon these moonlighting proteins and a very good example would be to analyse the three-dimensional structure of these proteins to superficially understand their molecular attributes.

Wanting more from our search for elements which could be targeted against *Cucumber mosaic virus* movement protein (CMV-MP), we came across the Glyceraldehyde Phosphate Dehydrogenase (GAPDH) which we fished out of *Cucumis Sativus*. Since its role was well elucidated as a potential moonlighting candidate, we hypothesised and empirically worked to check the interaction of the GAPDHs against the CMV-MP. In lieu of the above, our work focused on profiling of host genes showing interaction with CMV-MP (GAPDH in our study). Furthermore, GAPDH is a ubiquitous enzyme involved in glycolysis and the carbon reduction cycle of photosynthetic organisms. It also has non-glycolytic functions which include fusion of membranes, the exportation of nuclear RNA, the replication and repair of DNA, the bundling of microtubules, apoptosis, and viral pathogenesis. It also regulates the replication and translation of viruses as previous reports suggests, paving the way for its importance in understanding viral movement and pathogenesis. Plants contain several isoforms of GAPDH (GAPDH A, GAPDH B and GAPDH C) encoded by different types of genes (*GAP A*, *GAP B*, *GAP C* and *GAP Cp*) which are located in different sub-cellular compartments. *GAP A* and *GAP B* genes give rise to A_2B_2 - and A_4 -GAPDH isozymes of chloroplasts which participate in the Calvin-Benson cycle.

To transport the viral genome into adjacent cells, the viral movement proteins interact with the host proteins to increase the size exclusion limit of plasmodesmata. In another study performed in our laboratory, the association of a cell wall localised ascorbate oxidase from *Cucumis Sativus* with MP of CMV was reported. In a very crucial experiment using the GAL4-based yeast-two-hybrid (Y2H) system, we identified a GAPDH, one of the host proteins that interacted with CMV-MP in virus infected *Cucumis Sativus*. A gene fusion of GAPDH in pGADT7 and MP in pGBKT7 expression vector was generated and introduced in yeast strain AH109. Interaction between GAPDH and MP allowed the reconstitution of the DNA-binding and activation domains of the GAL4 transcription factor thereby activating the reporter genes which enabled the yeast to grow on nutrient selection medium. Different concentrations of 3-AT were added in the selection media to suppress the leaky expression of HIS3 gene and detect the positive clones. The interaction was also confirmed using colorimetric assay for β -galactosidase enzyme in the presence of X-gal which

resulted in the appearance of blue colonies of the yeast due to the activation of lacZ reporter gene.

The results were very promising as all the different isoforms of GAPDH showed a positive interaction with the virus MP. This investigation confirmed a hypothesis that pre-existing cellular factors within the host are imperative in participation with viral proteins to positively culminate into its spread and subsequent infection. As it is fairly well known, there are no sprays to stop the spread of viral infections in plants, antagonistic to the case of bacteria and fungi, exploring molecular mechanisms which participate in retarding the viral spread, is the best way currently known in virus spread impedance. We have proposed the need for genome editing in plants which could curate into an aspect, within plant immunity studies, to excavate possibilities in developing tolerance against ssRNA viruses which are majorly pathogenic to crop plants leading to millions of dollars' worth damage. Our Y2H study was preliminary, but it gave a high throughput analysis of the interactions that were checked and a confirmation that our search for plant agents conferring virus resistance was going on the right track.

An organism is majorly benefitted from the presence of multifunctional proteins as it reduces the number of synthesized proteins and also lessens the DNA required for replication. The amalgamation of the functionalities gives rise to various pathways to initiate, control and coordinate diverse cellular processes including starkly independent ones such as signalling and metabolism. These aspects of studies directly take part in plant immune system responses which can be studied *via* various omics approaches. Our laboratory, therefore, focuses on these aspects which can lead to crop improvement and usher in a phase of basic science research which, through plant factor manipulation, can control the viral pathogenesis and contribute to healthier and better varieties of important crops. Maybe, one day, our pursuits will lead us to crack the code on why viruses are so heartless in avenging and revenging, or maybe, just maybe, it is us the humans whose uncouth interventions have become competition of sorts to the viruses just to see who is more evolutionarily conserved and fit.

This, in short, summarises my research findings but in the early stages of my doctoral study, I really hope for something significant by the time the fruits of my hard labour ripens. I am being positive and optimistic with future goals and aims of doing something beneficial for the society at large in sight. I wish that by the end of it all I would be able to make my intellect useful to common people and fulfil my dream of being a proud and satisfied 'Plant Virologist!'