CHAPTER 5
YEAST TWO-HYBRID SCREENING TO IDENTIFY THE CANDIDATE HOST PROTEIN INTERACTIONS OF CHANDIPURA VIRUS MATRIX PROTEIN

5.1 INTRODUCTION

Yeast two-hybrid (Y2H) system is a very resourceful tool used for studying protein-protein interactions. Its feasibility and effectiveness in verifying known protein interactions and screening candidate proteins, which specifically interact with target proteins have been confirmed and extended to cell cycle regulation, signal transduction, drug discovery, and other virus-host interaction fields. Y2H system, first described by Fields and Song in 1989 [48], has been used extensively to study viral-host interactions of many viruses, including Varicella-Zoster Virus (VZV, family *Herpesviridae*) [152], Epstein Barr Virus (EBV, family *Herpesviridae*) [153], Pox Virus (PV, family *Poxviridae*) [154], Vaccinia Virus (VV, family *Poxviridae*) [155], Hepatitis C Virus (HCV, family *Flaviviridae*) [156], Hepatitis E Virus (HEV, family *Hepeviridae*) [157], Dengue Virus (DENV, family *Flaviviridae*) [158] and Influenza Virus (IV, family *Orthomyxoviridae*) [159]. It is based on the observation that the yeast GAL4 transcription factor possesses two separable domains, the N terminal DNA binding domain (BD) and a C terminal activation domain (AD) which activates the transcription of the downstream genes. BD contains a nuclear localization signal (NLS) and hence will be transported into the nucleus where it will bind to the upstream activating sequence (UAS) in a sequence specific manner. However, this domain fails to activate transcription. AD on the contrary, contains the activating regions which recruits the RNA polymerase, but cannot activate transcription because it fails to localize to the nucleus and also cannot bind to DNA. Y2H takes advantage of this property of the GAL4 transcription factor wherein both the domains are required for the induction of gene expression, but they do not need to be present within the same protein. Therefore, when two test proteins are fused with BD and AD, respectively, the two domains will be brought into proximity provided the test proteins can associate with each other, thus reconstituting a functional transcription factor and driving expression of downstream reporter gene.

This chapter focuses on screening the human fetal brain cDNA library to identify the candidate host proteins that interact with the M protein of CHPV using MATCHMAKER GAL4
two-hybrid system 3 (Clontech, USA). In this system, M proteins was expressed as a fusion with GAL4 DNA-BD (bait protein), while the human fetal brain cDNA library was expressed as fusion with GAL4 DNA-AD (prey protein). The screen was carried out under high stringency conditions to exclude the possibility of false positives. When the bait and prey proteins interact with one another, the DNA-BD and DNA-AD form an active transcription complex that can activate transcription of four downstream reporter genes, HIS3, ADE2, LacZ and MEL1. HIS3 and ADE2 provide strong nutritional selections for yeast cells that contain interacting proteins. MEL1 and LacZ encode α- and β-galactosidase, respectively, which provide additional colour indicators for isolation of positive yeast clones containing interacting proteins. This Y2H screening of cDNA library identified several host interactors of CHPV M protein.

5.2 RESULTS

5.2.1 Generation of BD Fusion Construct of Chandipura Virus Matrix Gene for Yeast two-hybrid Screening

Full length CHPV-M ORF was amplified from pUC plasmid clones containing CHPV-M gene (kindly provided by Prof. Dhrubajyoti Chattopadhyay, Kolkata, India). Primers (Table 5.1) flanked with restriction enzyme site at 5’ end were designed to facilitate in-frame cloning of M ORF (690 bps) with the DNA-BD sequence of the bait vector (pGBKT7) at its N-terminus. Following PCR, the amplicons were analysed on 1% agarose gel and were observed at its expected size after electrophoresis (Figure 5.1). The purified PCR product and pGBKT7 (BD) vector were digested with corresponding pair of restriction enzymes and ligated using T4 DNA ligase (Fermentas). The ligation mixture was transformed into E. coli DH5α cells and screened on LB agar plates containing 50 µg/ml kanamycin. Cloning was confirmed by isolating plasmid DNA from transformants followed by restriction digestion (Figure 5.2). Sequencing of potential positive clones was performed and a clone with M in-frame with the GAL4 BD was isolated for subsequent work. This new plasmid was termed BD-M.
Table 5.1: Oligonucleotides used for PCR amplification of Chandipura Virus Matrix gene
F is forward primer and R is reverse primer. The names of the restriction enzymes are in parenthesis and their recognition sequences in bold.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Primer</th>
<th>Sequence (5'---3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD-M</td>
<td>BD-M F (Nde I)</td>
<td>GTGACATATGCAAAGACTGAAGAAGTTTATAG</td>
</tr>
<tr>
<td></td>
<td>BD-M R (Sal I)</td>
<td>ACTGTCGACTCAATGACTTCTTAGAAATCAG</td>
</tr>
</tbody>
</table>

Figure 5.1: PCR amplification of Chandipura Virus Matrix gene
Chandipura Virus Matrix gene was amplified from pUC clone using gene specific primers for cloning in pGBKT7 vector. L is 1 kb DNA ladder (Fermentas).

Figure 5.2: Restriction enzyme digestion of BD-M
BD-M plasmid was isolated from E. coli DH5α cells and digested with restriction enzymes Nde I and Sal I. Only BD vector was taken as a control and digested with the same combination of enzyme as the recombinant construct. The linearization of the vector at ~ 7 kb and release of fallout corresponding to Matrix gene at ~ 690 bps confirmed cloning. L is 1 kb DNA ladder (Fermentas, USA).
5.2.2 Expression of BD-M in *Saccharomyces cerevisiae* strain AH109

Recombinant plasmid BD-M was transformed into competent *Saccharomyces cerevisiae* strain AH109 cells following the LiAc yeast transformation protocol and the transformants were selected on SD-Trp plates (nutritional selection for cells harboring BD plasmids, Figure 5.3). Following transformation, the cells were lysed and the total protein was extracted. The extracted protein profile was analysed by Western blotting. The immunoblotting with tag specific antibodies (anti c-myc antibodies for BD fusions) confirmed the expression of M protein as BD fusion (~ 54 kDa) in yeast cells (Figure 5.4).
5.2.3 Screening of Human Fetal Brain cDNA Library for Interactors of Matrix Protein

Subsequent to expression analysis, prior to Y2H screening, the positive transformants were analysed for autoactivation i.e. activation of *HIS3* reporter gene resulting from interaction of either BD fusion or AD fusion alone with transcriptional factors bound to TATA boxes leading to basal transcriptional activity. Autoactivation of BD-M fusion in AH109 cells was analysed by plating the transformants on SD -Trp/-His media. The absence of growth on these plates indicated that the BD fusion of M protein was not autoactivating.

Following autoactivation analysis, AH109 yeast cells harboring recombinant plasmid BD-M were mated with *Saccharomyces cerevisiae* strain Y187 cells, pre-transformed with human fetal brain cDNA library, at 30 °C for 24 hrs with shaking at 50 rpm. The diploid clones were plated on 40 SD -Trp/-Leu/-His (medium stringency/ triple dropout medium/TDO) plates of 150 mm diameter, each supplemented with X-α-Gal and were incubated at 30 °C for a fortnight. The absence of amino acids tryptophan and leucine in the medium enabled the selection of only those diploid clones which harbored both BD and AD plasmids. Likewise, the histidine deficient medium served as a nutritional selection for diploid clones which harbor bait and prey plasmids that code for proteins which interact with one another. Lastly, X-α-Gal aided in blue-white screening. A total of 425 colonies were isolated from the medium stringency plates which were subsequently streaked on high stringency/ quadruple dropout medium (QDO; SD -Trp/-Leu/-His/-Ade) supplemented with X-α-Gal and were further incubated at 30 °C for a week. The high stringency selection involved the depletion of yet another nutrient – adenine from the medium over and above the existing medium stringency conditions. A total of 150 colonies were isolated following selection on quadruple dropout and were categorized as high stringency clones. The colonies screened were labelled according to the number given to the TDO colony before they were re-streaked on QDO medium.

The high stringency clones were then analysed by yeast colony PCR (using AD specific primers) to determine the insert size of the prey plasmids as well as to screen for the presence of multiple prey plasmids. Following PCR, the analysis of the amplicons on 1 % agarose gel showed that the insert size ranged from 500 bps to 3 kb and that certain high stringency clones did contain multiple prey plasmids as indicated by multiple bands (Figure 5.5). The subsequent step was thus to eliminate them. The multiple prey plasmids were eliminated by re-streaking (sub culturing) the
corresponding diploid clone on SD -Trp/-Leu medium supplemented with X-α-Gal, repeatedly, and performing colony PCR (after each sub culture) until it was observed that the multiple prey plasmids segregated into two different daughter cells.

The next step was to determine whether the observed prey plasmid insert in each colony that had the same size across different colonies, was indeed the same insert (otherwise termed as ‘duplicate plasmids’) or whether they represented different clones from the library. This was done by restriction enzyme digestion of the PCR products with Hae III enzyme followed by the comparison of their restriction profiles on 0.8 % agarose gel (Figure 5.5). The digestion results indicated that most of the amplicons with same size from different colonies produced different digestion profile and thus did represent different clones from the library.

Following the elimination of multiple and duplicate prey plasmids, 50 out of the remaining 110 high stringency clones were selected for further analysis based on the rate of their growth on QDO media. These clones produced blue colonies within a week after streaking on QDO media supplemented with X-α-Gal and hence were considered as strong binders of the matrix protein. The prey plasmid was isolated from these selected clones by culturing them on SD -Leu media under a selective pressure to maintain pGAD-Rec cDNA library plasmids (AD/prey plasmid) within the yeast cells but with no pressure to retain BD-M (bait) plasmid. The isolated plasmids were transformed in E. coli DH5α cells followed by bacterial colony PCR to confirm the transformation of prey plasmids (Figure 5.6). The positive clones were sequenced using T7 forward and AD reverse primers.

5.2.4 DNA Sequencing

The T7 forward and AD reverse primers (Table 5.2) used for sequencing the prey plasmid inserts ensured that the sequence obtained contained informations of both the vector and the cDNA library insert. The SMART III oligonucleotide sequence (A AGC AGT GGT ATC AAC GCA GAG TGG; used for making cDNA clones) at the 5’ end of the sequence was taken as start point of the library insert while the CDS III sequence (CAT GTC GGC CGC CTC GGC CTC TAG A) at 3’ marked the end of the cDNA library insert.
Figure 5.5: Elimination of multiple and duplicate prey (library) plasmids.

(A) Yeast colony PCR of the blue coloured colonies that grew on QDO media supplemented with X-α-Gal was performed using T7 forward and AD reverse primers. The PCR products were analyzed on 1 % agarose gel. (B) The amplicons that had the same size (clone nos. 17, 21, 22 and 32 in 2A) across different colonies were digested with Hae III restriction enzyme to check whether they represented the same or different clones in the library. The inserts with same DNA sequence (duplicates) produced same digestion profile (clone nos. 22 and 32) while different inserts produced different profiles (clone nos. 17 and 21). (C) Multiple bands in PCR products (clone no. 16 and clone no. 35 in 3A) represented the presence of multiple prey plasmids in a single yeast cell. These clones were selected on DDO media (SD -Trp/-Leu [Synthetic Dropout media lacking amino acids tryptophan and leucine]) supplemented with X-α-Gal to segregate the prey plasmids. In certain cases the colonies retained their blue colour even after the prey plasmids were segregated (e.g., clone nos. 35a and 35b); while the others (e.g., clone nos. 16a and 16b) on contrary had two types of colonies - blue and white. Blue colonies represented interacting protein while white corresponded to clones harboring plasmids coding for non-interacting proteins and hence were discarded. The segregation of prey plasmids were confirmed by a second yeast colony PCR.
The prey plasmid isolated from yeast cells were transformed in *E. coli* DH5α cells. Transformation was confirmed by bacterial colony PCR using T7 forward and AD reverse primers. L is 1 kb DNA ladder (Fermentas, USA).

Table 5.2: Primers used for the sequencing cDNA library clones

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Primer</th>
<th>5’-------3’ sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T7 Forward</td>
<td>TAATACGACTCACTATAGGGC</td>
</tr>
<tr>
<td>2</td>
<td>AD reverse</td>
<td>AGATGGTGACACGATGCACAG</td>
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</tbody>
</table>

Following sequencing, the positive clones were subjected to a Blastn search on the human genomic and transcript database of National Centre for Biotechnology Information (NCBI). Blast identified 10 clones (Table 5.3) which were found to encode either complete ORF (3 clones) or a region of known human protein (7 clones) as potential candidate interactors of CHPV M protein. Rest of the sequences involved chromosome contigs, uncharacterized loci, cDNA inserts from a region outside the CDS of the gene or incorrect reading frame of the gene insert. The amino acid sequences of all the proteins encoded by the cDNA library inserts were determined using ExPASy (Expert Protein Analysis System) translation tool and were compared to the human protein sequences on the NCBI website using the Blastp.
Table 5.3: List of human proteins identified by Blastp analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Clone no.</th>
<th>Interacting Human Host Protein</th>
<th>Size of library clone (in aa)</th>
<th>Size of human protein (in aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MH104</td>
<td>ATP binding cassette, sub family E (OABP), member 1 (ABCE1)</td>
<td>599</td>
<td>599</td>
</tr>
<tr>
<td>2</td>
<td>MH122</td>
<td>CTD nuclear envelope phosphatase (CTDNEP1)</td>
<td>244</td>
<td>244</td>
</tr>
<tr>
<td>3</td>
<td>MH182</td>
<td>Developmentally regulated GTP binding protein 1 (DRG1)</td>
<td>367</td>
<td>367</td>
</tr>
<tr>
<td>4</td>
<td>MH216</td>
<td>Kinesin family member 5A (KIF5A)</td>
<td>341</td>
<td>1032</td>
</tr>
<tr>
<td>5</td>
<td>MH231</td>
<td>ss DNA binding protein 2 (SSBP2)</td>
<td>309</td>
<td>369</td>
</tr>
<tr>
<td>6</td>
<td>MH323</td>
<td>THO complex 2 (THOC2)</td>
<td>327</td>
<td>1593</td>
</tr>
<tr>
<td>7</td>
<td>MH365</td>
<td>Transient receptor potential cation channel, sub family C, member 4 associated protein (TRPC4AP)</td>
<td>164</td>
<td>797</td>
</tr>
<tr>
<td>8</td>
<td>MH52</td>
<td>Transmembrane protein 45A (TMEM45A)</td>
<td>215</td>
<td>275</td>
</tr>
<tr>
<td>9</td>
<td>MH76</td>
<td>Tubulin alpha 1b (TUBA1B)</td>
<td>410</td>
<td>451</td>
</tr>
<tr>
<td>10</td>
<td>MH79</td>
<td>Tubulin beta 1 (TUBB)</td>
<td>123</td>
<td>444</td>
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</table>
5.2.5 Alignment and Description of Clones Identified

5.2.5.1 Protein Sequence Blast Search of Clone MH52

Blastp analysis showed that clone MH52 encodes human Transmembrane protein 45A (TMEM45A). This protein consists of 275 aa and the cDNA insert encoded the N terminal 215 aa. The alignment between query and subject showed 100% identities with no gap (Figure 5.6).

The protein blast analysis of clone MH52 identified that the cDNA insert encoded human Transmembrane protein 45A protein.

Figure 5.7: Blastp analysis of clone MH52
**5.2.5.2 Protein Sequence Blast Search of Clone MH76**

Blastp analysis showed that clone MH76 encodes human Tubulin alpha 1b (TUBA1B). This protein consists of 451 aa and the cDNA insert encoded a N terminal Δ42 truncate. The alignment between query and subject showed 100% identities with no gap (Figure 5.7).

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**Figure 5.8: Blastp analysis of clone MH76**

The protein blast analysis of clone MH76 identified that the cDNA insert encoded human Tubulin alpha 1b protein.
5.2.5.3 Protein Sequence Blast Search of Clone MH79

Blastp analysis showed that clone MH79 encodes human kinesin family member 5A protein (KIF5A). This protein consists of 1032 aa and the cDNA insert encoded the C terminal 341 aa. The alignment between query and subject showed 100% identities with no gap (Figure 5.8).

Figure 5.9: Blastp analysis of clone MH79
The protein blast analysis of clone MH79 identified that the cDNA insert encoded human Kinesin family member 5A protein.
5.2.5.4 Protein Sequence Blast Search of Clone MH104

Blastp analysis showed that clone MH104 encodes human ATP binding cassette, sub-family E (OABP), member 1 (ABCE1) protein. This protein consists of 599 aa and the cDNA insert encoded the full length protein. The alignment between query and subject showed 100% identities with no gap (Figure 5.9).

**Figure 5.10:** Blastp analysis of clone MH104

The protein blast analysis of clone MH104 identified that the cDNA insert encoded human ATP binding cassette, sub-family E (OABP), member 1 (ABCE1) protein.
5.2.5.5 Protein Sequence Blast Search of Clone MH122

Blastp analysis showed that clone MH122 encodes human CTD Nuclear Envelope Phosphatase 1 (CDTNEP1). This protein consists of 244 aa and the cDNA insert encoded the full length protein. The alignment between query and subject showed 100 % identities with no gap (Figure 5.10).

![CTDNEP1](image)

**Figure 5.11:** Blastp analysis of clone MH122

The protein blast analysis of clone MH122 identified that the cDNA insert encoded human CTD Nuclear Envelope Phosphatase 1 protein.
5.2.5.6 Protein Sequence Blast Search of Clone MH182

Blastp analysis showed that clone MH182 encodes human developmentally regulated GTP binding protein 1. This protein consists of 367 aa and the cDNA insert encoded the full length protein. The alignment between query and subject showed 100% identities with no gap (Figure 5.11).

**Figure 5.12:** Blastp analysis of clone MH182

The protein blast analysis of clone MH182 identified that the cDNA insert encoded human developmentally regulated GTP binding 1 protein.
5.2.5.7 Protein Sequence Blast Search of Clone MH216

Blastp analysis showed that clone MH216 encodes human Tubulin beta 1 (TUBB). This protein consists of 444 aa and the cDNA insert encoded C terminal 123 aa. The alignment between query and subject showed 100% identities with no gap (Figure 5.12).

![Figure 5.13: Blastp analysis of clone MH216](image)

The protein blast analysis of clone MH216 identified that the cDNA insert encoded human Tubulin beta 1 protein.
5.2.5.8 Protein sequence Blast search of clone MH231

Blastp analysis showed that clone MH231 encodes human THO Complex 2 (THOC2). This protein consists of 1593 aa and the cDNA insert encoded N terminal 327 aa. The alignment between query and subject showed 98% identities with no gap (Figure 5.13).

**Figure 5.14:** Blastp analysis of clone MH231

The protein blast analysis of clone MH231 identified that the cDNA insert encoded human THO Complex 2 protein.
**5.2.5.9 Protein Sequence Blast Search of Clone MH323**

Blastp analysis showed that clone MH323 encodes human Transient receptor potential cation channel, subfamily C, member 4 associated protein (TRPC4AP). This protein consists of 797 aa and the cDNA insert encoded C terminal 164 aa. The alignment between query and subject showed 100% identities with no gap (Figure 5.14).

**Figure 5.15:** Blastp analysis of clone MH323

The protein blast analysis of clone MH323 identified that the cDNA insert encoded human Transient receptor potential cation channel, subfamily C, member 4 associated protein.
5.2.5.10 Protein Sequence Blast Search of Clone MH365

Blastp analysis showed that clone MH365 encodes human Single strand DNA binding protein 2 (SSBP2). This protein consists of 369 aa and the cDNA insert encoded N terminal 309 aa. The alignment between query and subject showed 100% identities with no gap (Figure 5.15).

SSBP2
Sequence ID: lcl|196437  Length: 369  Number of Matches: 2

<table>
<thead>
<tr>
<th>Range 1: 1 to 309</th>
<th>Graphics</th>
<th>Method</th>
<th>Identities</th>
<th>Positives</th>
<th>Gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 612 bits(1578) 0.0</td>
<td>Compositional matrix adjust.</td>
<td>309/309(100%)</td>
<td>309/309(100%)</td>
<td>0/309(0%)</td>
<td></td>
</tr>
</tbody>
</table>

Query 1
MYGKGKNSSSAVPSDSQAREKLALYYVEYLLHVGQKSAQTFSLSIERWEEKTILLPGEFGF 60
Query 61
LHSWCVLIdyCAAPPERETCEHSEAKAFHDYSAAAASPVLGNIIPDGKMPGPVPP 120
Query 121
GFPQPPFMSPYPPGPRPLRIPNALGGPGSQPLPSGIIDPQGPGNPYNPGQHTNHTP 180
Query 181
PRGMLPGQSDWLSLQNYGGAIIRPPLNLAGHRGPMGNNPNPGGPRPUPNPTANIPSY 240
Query 241
SSASPNVYVPPGDPGPPPTPGMPSPADSTNSGNYTLYINAVPPGPNRPNPMPGSDG 300
Query 301
PMKGLGGMIE 309

SBjct 1
MYGKGKNSSSAVPSDSQAREKLALYYVEYLLHVGQKSAQTFSLSIERWEEKTILLPGEFGF 60
SBjct 61
LHSWCVLIdyCAAPPERETCEHSEAKAFHDYSAAAASPVLGNIIPDGKMPGPVPP 120
SBjct 121
GFPQPPFMSPYPPGPRPLRIPNALGGPGSQPLPSGIIDPQGPGNPYNPGQHTNHTP 180
SBjct 181
PRGMLPGQSDWLSLQNYGGAIIRPPLNLAGHRGPMGNNPNPGGPRPUPNPTANIPSY 240
SBjct 241
SSASPNVYVPPGDPGPPPTPGMPSPADSTNSGNYTLYINAVPPGPNRPNPMPGSDG 300
SBjct 301
PMKGLGGMIE 309

Figure 5.16: Blastp analysis of clone MH365

The protein blast analysis of clone MH365 identified that the cDNA insert encoded human Single strand DNA binding protein 2.

5.3 DISCUSSION

The rhabdovirus matrix (M) protein is a multifunctional virion protein that plays major role in virus assembly and budding, virus-induced inhibition of host gene expression and cytopathic effects observed in infected cells. The myriad roles played by this protein in the virus biology makes it a critical player in viral pathogenesis. Therefore, discerning the interactions of this protein with host can greatly facilitate our understanding of virus infections, ultimately leading to both improved therapeutics and insight into cellular processes. The aim of this chapter
was to screen the human fetal brain cDNA library for interactors of CHPV M protein using Y2H system as the method of choice. Y2H system is a powerful tool to screen the direct interaction of host protein with the specific viral protein of interest [48]. This system allows in vivo characterization of protein associations in cases where biochemical experiments such as co-purification, was found to be unsuccessful [160, 161, 162], because of the kinetic parameter constraints [163].

Y2H screening successfully identified ten human host proteins that can interact with the M protein of CHPV. The interactions were confirmed by more than three rounds of testing in yeast under high-stringency conditions to eliminate false positives. The identified host proteins are part of various cellular machineries, pathways and processes which are circumvented to perform varied functions at different stages of virus life cycle which in turn emphasize the dynamic nature of M protein. The functional relevance of these interactions at various junctures of viral pathogenesis can be speculated as follows:

(a) **Viral transcription**

Cellular Cytoskeleton protein tubulin is one of the host proteins identified to interact with CHPV M protein in the present study. Research on Sendai Virus (SeV, Family Paramyxoviridae) has shown that tubulin (both alpha and beta subunits) activates transcription of viral RNA by dissociating M protein from the ribonucleoprotein (RNP) core. M protein maintains the RNP in the condensed form and hence acts as a negative regulator for transcription. During disassembly the M protein directly interacts with tubulin and is released from the RNP as a complex. These findings suggest that interaction of M protein with tubulin may have an important role in the regulation of CHPV transcription.

(b) **Virus assembly and budding**

Studies on Influenza A Virus (IAV, Family Orthomyxoviridae) have shown that the acetylated microtubules (tubulin and actin) play crucial roles in polarized trafficking of viral components to apical plasma membrane during virus assembly [164]. Human Immunodeficiency Virus (HIV, Family Retroviridae) assembly and budding take place within a lipid raft-rich platform that appears to be constrained and/or maintained by the actin and tubulin cytoskeleton. Disruption of either actin or tubulin remodeling dispersed this platform, resulting in reduced virus spread, virion release and viral infectivity. Although its association with M protein has not been studied, human ABCE1 protein has previously been shown to be essential as the major source of
energy during the assembly of HIV [165] and Rabies Virus (RV, Family Rhabdoviridae) [166] capsid proteins. This protein is also known to associate with the virion of prototype rhabdovirus VSV [167].

(c) Cytopathic effects – apoptosis, cell rounding and virus-induced inhibition of host gene expression

The over expression of the host protein CTDNEP1, found to interact with M protein in this study, has been shown to result in apoptosis [168]. The N terminal 45 amino acids of CTDNEP1, behaves as a nuclear localization signal (NLS) and is capable of targeting the bound protein to the nuclear membrane [169]. Since CHPV M protein does not have any NLS, this interaction might be aiding the viral protein to reach the nuclear membrane, where it is known to associate with the nuclear pore complex and proteins like THOC2 (required for efficient export of polyadenylated RNA and spliced mRNA) to inhibit the nucleo-cytoplasmic transport of host mRNAs [9].

(d) Virus spread in the Central Nervous System

Subsequent to cerebral invasion, CHPV could spread among neuronal populations by exploiting the synaptic pathways like VSV [170], Herpes Simplex Virus (HSV, family Herpesviridae) [171] and RV [172], as suggested by the interactions of the viral proteins with host motor proteins, such as kinesin, dynein (DYN) and/or dynactin (DCTN) either directly or through accessory proteins. Kinesin family member 5A protein (KIF5A) identified to interact with the M protein of CHPV in the present study, aids in the plus end directed anterograde neuronal transport of the bound cargo [144]. The functional relevance of the interaction among CHPV M protein and KIF5A has been proposed based on the leading model available in the literature which explains the transport of viruses within infected cells. The model suggests that, the viral capsids and glycoproteins are transported separately, and assembled somewhere along the axon shaft or at the axon terminus [reviewed in 173 and 174].

It was expected that both the in-silico and Y2H studies would identify common host protein interactors for M protein. At least this was the case for CHPV G protein (unpublished work) or in the case of Chikungunya virus (CHIKV) envelope proteins [175] where both the studies identified common interactors. The protocols followed in all these studies were exactly same for both the viruses.
In summary, the analysis of the human-virus protein-protein interaction network thus provides a framework to gain insights into the molecular mechanisms underlying the hijacking of host cell machinery by viruses for their perpetuation.