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# Role of Viral Protein-Protein Interactions and Possible Targets for New Therapeutics

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## Abstract

Viral infections are the cause of serious infirmities in humans and kill millions of people every year. Management of viral diseases is one of the challenges faced by the whole world which needs improvement in prevention and treatment options. Complete understanding of the consequences of viral proteins interaction network on host physiology is essential. Towards this goal, deciphering viral protein-protein interactions is one of the perspective which can help in our understanding about the basis of viral pathogenesis and the development of new antivirals. Indeed, viral infection network based on viral-viral proteins will provide an elusive and investigative framework to articulate rationalize drug discovery based on proteomics scale of viruses. In this study, proteomics a collection of viral-viral protein interactions reporting different studies of hepatitis C virus, Influenza A virus, Dengue virus and SARS Coronavirus. Our effort of protein-interactions was focused on different studies reporting interactions between viral proteins encoded by the viruses under study. The study is integrated with a broad and original literature-curated data of viral-viralprotein (197 non-redundant) interactions.

## Introduction

Viruses interfere vital cellular processes, including cell growth, gene expression, differentiation and signalling pathways by perturbing the networks of cellular regulatory processes. Alongside, the capture of cellular machinery by viruses for the production of their own progenies and evasion from the host immune system is the major cause of pathogenesis. Mechanisms essential for this sabotage of cellular physiology facilitated by viral proteins can be understood only by uncovering that how viral proteins disturb normal cell signalling networks along with the significance of intra-play of viral protein-protein interactions.

Viral protein–protein interactions are necessary for the fundamentals of virion assembly and its egression from the cell. Often, these interactions cause functional perturbations which may lead to complex diseases, like cancer(Ahmed and Heslop, 2006; Hebner and Laimins, 2006).

In this context, we have reported the analysis and combination of viral protein-protein interactions. Based on an extensive scientific literature search, we provided a resource of manually curated interactions between viral proteins.

## Hepatitis C Virus (HCV)

Since the discovery of this virus as the causative agent of hepatitis(previously known as Non-A, Non-B hepatitis) (Choo *et al*., 1990), significant improvement has been made in understanding of the molecular biology of virus and its replication pathways inside the cell. However, the progress has been hindered by non-efficient cell culture system of virus (Barth *et al*., 2006). HCV is a member of Flaviviridae family. It is an enveloped, positive-strand RNA virus with 9.6kb genome (Rice, 1996). Un-translated region (UTR) is present at 5' and 3' ends, which flanks single poly protein consisting of 3010 amino acids. It is further processed into 4 structural proteins i.e. core (C) , Envelope (E1, E2) and p7 along with 6 non-structural (NS) proteins comprising NS2, NS3, NS4A, NS4B, NS5A and NS5B. These proteins are post processed by host and viral proteases(Lohmann *et al*., 1996) for their cleavage as individual functional proteins. HCV genome gets translated into a large polypeptide that has to be cleaved into appropriate proteins to induce viral replication. In a study, it was observed that at the N-terminus of NS3 is a serine protease that cleaves the polypeptide at four distinct regions i.e. NS3/NS4A, NS4A/ NS4B, NS4B/ NS5A and NS5A/ NS5B. NS4A forms the integral part of NS3 and this association is strongly mediated by Isoleucine (Ile)-29 of NS4A. In addition, two residues of NS4A i.e. Ile-25 and Val-23 are involved in developing its association with NS3 as well as with NS4B and NS5A (Dimitrova *et al*., 2003).

Structural proteins C, E1and E2 serve as structural component and play their part in the assembly of virions (Bartosch *et al*., 2003; Nielsen *et al*., 2004; Yasui *et al*., 1998). P7 is a small protein and considered to be a membrane channel protein(Carrere-Kremer *et al*., 2002). Non-structural proteins consist of NS2–NS5B.Their proteolytic cleavage is mediated by two viral proteases. NS2-NS3 protease, which cleaves NS2 from the poly protein. NS3 serine protease along with its cofactor 4A mediates the cleavage of other proteins of virus. N-terminus of NS3 constitutes serine protease domain while its C-terminus consist of RNA Helicase domain. NS4A acts as a cofactor for NS3 serine protease (Tellinghuisen and Rice, 2002). NS4B function is still not clear but it is proposed that it is associated with membranes and helps in

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replication of virus. NS5A is hydrophilic membrane associated protein whose function is unknown, until recently genetic evidence has been provided that in the phase of HCV RNA replication, NS5A performs two distinct functions. A cis-acting and other one is trans-acting function. Cis-acting happens as part of the HCV replication complex, and trans-acting function may occur outside of the replication complex. It was confirmed by an inhibitor of NS5A, BMS-790052(Fridell *et al*., 2011). The NS5B protein is the RNA-dependent RNA polymerase, helping in the replication of the virus.

Several studies have shown that viral non-structural proteins are also involved in replication, virion assembly and its maturation, especially in case of Flaviviridae family (Kummerer and Rice, 2002; Liu *et al*., 2003). NS proteins interact with themselves and structural proteins during viral lifecycle. These interactions are of significant values in terms of development of specific inhibitors by analysing the residues present at the interface which are involved in interaction.

Several studies report the interactions among HCV proteins. Here provided a review of these interactions reported in literature showing the interactions among viral proteins and their significance in relation to viral replication and assembly.

Interaction between the Core protein and the helicase portion of NS3 has been confirmed recently. The protease portion of NS3 does not play any role in it. This interaction was confirmed by four different biochemical methods. Protein-protein interaction could be interrupted by two types of inhibitors including SL201 and core106 which is a truncated core protein from C-terminus. Cross-linking experiments suggested that the interaction of core-NS3h is most likely driven by C protein oligomerization. SL201 is basically involved in the production blockage of infectious virus, and does not take part in the production of subgenomic HCV replicon. Experiments have also articulated that SL201 has no role in the inhibition of virus entry. This data reveals the importance of core and viral helicase interaction in the assembly of virion (Mousseau *et al*., 2011).

HCV core protein forms a nucleocapisd that gets surrounded by a lipid bilayer. The mutational analysis of HCV core protein has been determined that indicates that hydrophilic domain (1-115) of this protein is critically involved in its multimerziation which, in turn, is involved in various functions of the core protein (Matsumoto *et al*., 1996). In order to determine the core-core protein interaction, several truncations were done. This led to the findings that a tryptophan rich sequence region ranging from 82-102 in the N-terminal region is important for homotypic interactions while C-terminus hydrophobic region shows a weak heterotypic interaction with N-terminal region of the core protein (Nolandt *et al*., 1997).

In addition to the interaction of core protein with itself and some other glycoproteins of the virus. It also displays interactions with the RNA dependant RNA polymerase i.e. NS5B, as determined by immunoflourescence and immunoprecipitation assays. These two proteins co-localizes onto the ER membrane. However, the C-terminal region of NS5B is critically involved in the complex formation between these two proteins as the truncated NS5B is vulnerable to establish a core protein-NS5B complex(Uchida *et al*., 2002).

NS5B forms a complex with NS3 region in the presence or absence of NS4A (cofactor for NS3 serine protease). It was shown that amino terminal of NS3 is responsible for the interaction with NS5B as both proteins were co-localized, proved by double staining analysis. It also revealed that NS4A does not interfere with NS5B and NS3 interaction. Co-immunoprecipitation assays proved that NS5B also forms a complex with NS4A in the absence of NS3. These results suggested that NS5B, NS3 and NS4A form a complex which might function as a part of replication machinery (Ishido *et al*., 1998).

NS5A modulates the HCV replication being a part of the replication complex. It was established by glutathione S-transferase (GST) pull-down and Co-immunoprecipitation assays that bacterial recombinant NS5A interacts with NS5B. Two regions,105–162and 277–334, were found to be important for their interactions (Shirota *et al*., 2002).

The strongest protein interactions among viral proteins have been found for NS3 with itself. Yeast two hybrid system was used to find these interactions. Minimal region of 174 amino acids at N-terminus of the helicase region was required for interaction which was validated by randomly introducing mutations and then narrowing down the functional interactions. The association of NS3 with itself was confirmed by Co-immunoprecipitation assays. Other parts of the NS3 protein subsidise to the stability of the NS3-NS3 interactions (Khu *et al*., 2001).

X-Ray crystallographic structure of NS5A has revealed the dimer formation for this protein in two crystal structures; NS5A (33-202) or NS5A (25-215). Both dimer structures support the concept of membrane-bound NS5A proteins associating through contact of domain I surfaces. Further studies are needed to elucidate the role of these dimer formations in HCV lifecycle (Love *et al*., 2009).

Folding and dimerization analysis of E1 and E2 proteins revealed that E1 folding is more efficient and faster than E2. E2 starts it's folding after getting associated with E1 protein. Co-immunoprecipitation and sedimentation rate analysis verified the association between these two proteins which exist as a non-covalent heterodimer, a functional subunit of HCV virion Envelope (Brazzoli *et al*., 2005).

The structural genes of HCV encode three proteins i.e. Core (C), two envelope proteins (E1) and (E2). Immunoprecipitation experiments revealed that Core protein interacts with the E1 protein in the presence of anti-core antibody. These proteins co-localize on the cytosolic region of endoplasmic reticulum (ER) membrane where E1 faces the core protein. In another study, core and glycoproteins of HCV (E1 and E2/NS1) was inserted into baculovirus expression system and each protein was inserted into a separate construct. The infected insect cells showed that core protein was phopshorylated and transported into nucleus. Deletion of C-terminal region containing hydrophobic residues, E2 protein was produced as soluble protein but not the secreted one. Contrary, there

**Table 1**. List of HCV protein-protein interactions.

| S # | Viral Protein 1 | | Viral Protein 2 | |
|---|---|---|---|---|
| | NCBI Reference Sequence | Protein | NCBI Reference Sequence | Protein |
| 1. | NP_751919 | core protein | NP_751919 | core protein |
| 2. | NP_751919 | core protein | NP_751920 | E1 protein |
| 3. | NP_751919 | core protein | NP_751927 | NS5A protein |
| 4. | NP_751919 | core protein | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 5. | NP_751920 | E1 protein | NP_751921 | E2 protein |
| 6. | NP_751923 | NS2 protein | NP_751923 | NS2 protein |
| 7. | NP_751923 | NS2 protein | NP_751925 | NS4A protein |
| 8. | NP_751923 | NS2 protein | NP_751926 | NS4B protein |
| 9. | NP_751923 | NS2 protein | NP_751927 | NS5A protein |
| 10. | NP_751923 | NS2 protein | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 11. | NP_751923 | NS2 protein | NP_803144 | NS3 protease/helicase |
| 12. | NP_751925 | NS4A protein | NP_751925 | NS4A protein |
| 13. | NP_751925 | NS4A protein | NP_751926 | NS4B protein |
| 14. | NP_751925 | NS4A protein | NP_751927 | NS5A protein |
| 15. | NP_751925 | NS4A protein | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 16. | NP_751926 | NS4B protein | NP_751926 | NS4B protein |
| 17. | NP_751926 | NS4B protein | NP_751927 | NS5A protein |
| 18. | NP_751926 | NS4B protein | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 19. | NP_751926 | NS4B protein | NP_803144 | NS3 protease/helicase |
| 20. | NP_751927 | NS5A protein | NP_751927 | NS5A protein |
| 21. | NP_751927 | NS5A protein | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 22. | NP_751927 | NS5A protein | NP_803144 | NS3 protease/helicase |
| 23. | NP_751928 | NS5B RNA-dependent RNA polymerase | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 24. | NP_803144 | NS3 protease/helicase | NP_751925 | NS4A protein |
| 25. | NP_803144 | NS3 protease/helicase | NP_751928 | NS5B RNA-dependent RNA polymerase |
| 26. | NP_803144 | NS3 protease/helicase | NP_803144 | NS3 protease/helicase |
| 27. | NP_751921 | E2 protein | NP_803144 | NS3 protease/helicase |
| 28. | NP_751920 | E1 protein | NP_751927 | NS5A protein |

is no effect of deleting C-terminal region of E1 on its solubility (Lo *et al*., 1996).

HCV replicates itself onto the ER membrane in the presence of viral assembly mainly consisting of all non-structural proteins. It was demonstrated that all of the HCV NS proteins get sequestered on the cytosolic membrane of ER and produces a high concentration of viral proteins, sufficient to induce pathogenesis in vitro as well as in vivo by using Glutathione pull down assay and co-immunoprecipitation assays(Dimitrova *et al*., 2003).

Previous studies (Dimitrova *et al*., 2003; Drummer and Poumbourios, 2004; Dubuisson *et al*., 1994; Flajolet *et al*., 2000; Goh *et al*., 2001; Khu *et al*., 2001; Lanford *et al*., 1993; Lim *et al*., 2006; Lin *et al*., 1997; Lo *et al*., 1996;

Ma *et al*., 2002; Matsumoto *et al*., 1996; Molenkamp *et al*., 2003; Nolandt *et al*., 1997; Op De Beeck *et al*., 2000; Piccininni *et al*., 2002; Shirota *et al*., 2002; Uchida *et al*., 2002; Welsch *et al*., 2007; Yan *et al*., 1998) have provided valuable data on the HCV protein-protein interactions listed in Table1.

## Dengue Virus

Dengue virus belongs to the family Flaviviridae that has seven non-structural proteins i.e NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Dengue virus has an RNA dependant RNA polymerase (NS5) that specifically interacts with its own Non-structural protein-3 (NS3), a helicase. These multi-domain proteins show interactions both *in vitro* and *in vivo*. However, NS3 has to compete with a cellular protein i.e. nuclear transport receptor importin–β to interact with N-terminal region of NS5 (residue 320-368). By using two hybrid system, it was observed that the virally infected cells hyperphosphorylate NS5 that preferentially interacts with nuclear localization signals like nuclear transport receptor importin β but not with NS-3. Later, the competition between NS3 and importin –β for NS5 was confirmed by using pull down assays that depicted the potential role of importin β to pull down cytoplasmic proteins to nucleus during viral infection (Johansson *et al*., 2001).

The NS3 protein of the Dengue virus has strong interactions with other NS proteins of the virus to facilitate its own helicase activity and favour viral replication. In this study, the interaction between NS3 and NS4B was identified by using two hybrid system followed by the confirmation with pull down assay. It was observed that NS-4B helps NS-3 to detach from ssRNA and improve its helicase activity *in vitro* (Umareddy *et al*., 2006).

To unravel the structural organization of flaviruses like Dengue virus and yellow fever virus, their capsid proteins were characterized. The secondary structure of their capsid proteins revealed large number of alpha helices at the C-terminus with the largest alpha helix of 20 residues. In addition, C-terminus is amphipathic while N-terminal region was not shown to be involved in structural integrity to the capsid protein (Jones *et al*., 2003).

The core region of Dengue virus type- 2 is protected with nucleocapsid region which, in turns, is surrounded with a lipid bilayer derived from the membrane (M) and an envelope protein. The mutational analysis of this membrane protein revealed that Histidine (H) at 39 position plays a pivotal role in viral replication, morphogenesis, cell entry and secretion (Pryor *et al*., 2004).

In a study, the mechanism of entry for Dengue virus (type-2) into the host cells was determined and observed that NS1 has two glycosylation sites i.e. Asn 130 and Asn 207. By inserting mutagenized cDNA into the Simian virus 40, it was observed that removal of one or both of these glycosylation sites can't abolish the dimerization and secretion of NS-1 yet secretion is greatly reduced by removing Asn 207 (Pryor and Wright, 1994).

The large genome of Dengue virus gets translated into multiple proteins that interact with each other to facilitate viral entry, replication and host specific pathogenesis. In order to find out the reason for dengue virus infection in humans but not in mosquitoes, antiNS-3 antisera was used that favoured the formation of NS-3 fragment of 50 kDa size with the N-terminal region containing 460 residues. This fragment is only formed in the dengue virus infected human but not in the mosquitoes. Further examination of this fragment revealed that there is conserved cleavage sequence within the helicase domain of NS-3, which is

**Table 2**. List of Dengue virus protein-protein interactions.

| | Viral Protein 1 | | | Viral Protein 2 | | |
|---|---|---|---|---|---|---|
| **S#** | **Virus Type** | **NCBI Reference Sequence** | **Protein** | **Virus Type** | **NCBI Reference Sequence** | **Protein** |
| 1 | Dengue virus type 1 | NP_722460 | envelope protein | Dengue virus type 1 | NP_733807 | membrane glycoprotein precursor |
| 2 | Dengue virus type 2 | NP_739587 | ATPase | Dengue virus type 2 | NP_739590 | NS5 |
| 3 | Dengue virus type 2 | NP_739589 | ns4b protein | Dengue virus type 2 | NP_739587 | ATPase |
| 4 | Dengue virus type 2 | NP_739591 | capsid protein (C) | Dengue virus type 2 | NP_739591 | capsid protein (C) |
| 5 | Dengue virus type 2 | NP_739582 | prM (M) protein | Dengue virus type 2 | NP_739583 | E protein |
| 6 | Dengue virus type 2 | NP_739584 | NS1 protein | Dengue virus type 2 | NP_739584 | NS1 protein |
| 7 | Dengue virus type 2 | NP_739586 | ns2b protein | Dengue virus type 2 | NP_739587 | ATPase |

cleaved by NS-2 and favours the viral replication and disease progression (Arias *et al*., 1993).

Previous studies (Arias *et al*., 1993; Brooks *et al*., 2002; Clum *et al*., 1997; Courageot *et al*., 2000; Johansson *et al*., 2001; Jones *et al*., 2003; Kuhn and Rossmann, 1995; Kuhn *et al*., 2002; Leung *et al*., 2001; Lindenbach and Rice, 1999; Ma *et al*., 2004; Niyomrattanakit *et al*., 2004; Preugschat and Strauss, 1991; Pryor *et al*., 2004; Pryor and Wright, 1994; Rey *et al*., 1995; Umareddy *et al*., 2006; Wang *et al*., 2004; Welsch *et al*., 2007) have provided valuable data on the Dengue virus protein-protein interactions listed in Table 2.

## Influenza A Virus

Influenza virus A (IAV) is a member of orthomyxoviridae family. The IAV genome is 13.5kb long single stranded negative sense RNA that constitutes 11 proteins. Two surface glycoproteins; HA (haemagglutinin) and NA (neuraminidase), Non-structural proteins; non-structural 1 (NS1), non-structural 2 (NS2), matrix 1 (M1), matrix 2 (M2), nucleoprotein (NP) and three polymerase proteins; PB1(polymerase basic protein 1), PB2, and PA (polymerase acidic protein), some members also produce PB1-F2, a small protein (Ghedin *et al*., 2005). Based on the antigenic nature of surface glycoproteins HA and NA, several subtypes of IAV have been characterized. These proteins are responsible for the most antigenic variations in the virus. So far, 16 different subtypes of HA (H1-16) and 9 different subtypes of NA (N1-9) have been identified (Fouchier *et al*., 2005).

The optimum propagation of IAV in the upper respiratory tract of humans has been seen at the temperature of 33°C and this temperature is 41°C in the intestinal tract of birds. The adaptation of virus to the host is performed by the viral RNA polymerase complex comprises of PA, PB1 and PB2. The activity of IAV RNA polymerase is optimum under the temperature control of PB2.PB1 interacts with PB2 either in the presence or absence of polymeraseacidic protein. Co-expression and co-immunoprecipitation assays have proved this association by using monospecific antibodies. Two regions at the NH2 terminus of PB1 have been found, which can interact in an independent manner with PB2 to form stable complexes. C-terminal of PB1 is not involved in making any interaction with PB2. Mutational analysis further established that the interacting regions of PB1 include amino acids (aa) 48-145 and 251-321 (Biswas and Nayak, 1996).

In another study, results suggested that the 78 aa of N-terminal and residues 506-659 of PB1 were interactively involved with compartments of polymerase. Mammalian cell two-hybrid assays has also proved that N-terminal of PB1 is responsible for its interaction with the PA subunit while the PB1 C-terminal region is responsible for binding with PB2 subunit (Gonzalez *et al*., 1996). No direct interaction between PA and PB2 has been confirmed, but a three dimensional structure of a recombinant influenza virus RNP created by electron microscopy proposed that contacts among the three polymerase subunits were present (Area *et al*., 2004). The effect of PA on the nuclear accumulation of PB1 was studied using Co-expression analysis. It was suggested that PB1 must interact with PA for its efficient nuclear accumulation (Fodor and Smith, 2004).

Some other interactions reported in studies (Biswas *et al*., 1998; Biswas and Nayak, 1996; Gonzalez *et al*., 1996; Hara *et al*., 2003; Marion *et al*., 1997; Mayer *et al*., 2007; Mazur *et al*., 2008; Ohtsu *et al*., 2002; Perez and Donis, 1995; Perez and Donis, 2001; Shapira *et al*., 2009; Sugiyama *et al*., 2009) have provided valuable data on the influenza protein-protein interactions listed in Table 3.

## Severe Acute Respiratory Syndrome (SARS)

Severe acute respiratory syndrome (SARS) is an infectious human respiratory disease caused by SARS Coronavirus (HCoVs). It was first discovered in a China town Guangdong where it caused havoc in an outbreak of a typical pneumonia in 2003 and quickly spread to 23 countries. The virus was identified as a noval coronavirus and was designated to the subfamily of Coronavirinaeorder Nidovirales (Drosten *et al*., 2003). The mortality rate due to the severity of the disease was reported to be ~3 to 6% at that time (Marra *et al*., 2003).The genome of HCoV, is an enveloped positive-sense, single-stranded RNA of nearly 30 kb (He *et al*., 2004). It is transcribed into two large polyproteins pp1a and pp1abwhich upon cleavage by virally encoded proteases gives non-structural proteins such as RNA-dependent RNA polymerase (Rep), an adenosine triphosphatase (ATPase) helicase (Hel) and16 other functional non-structural proteins (nsps) (Ziebuhr *et al*., 2000). The non-structural proteins then process viral replication as well as viral proteins organization. The most important structural proteins include envelope protein E, nucleocapsid protein and membrane proteins S (Spike) and M (membrane).

Identification of interacting amino acid sequences involved in protein-protein interaction are necessary for the elucidation of the SARS-CoV replication mechanism and thus execution of anti-SARS therapeutic interference.

The nucleocapsid protein of 46 kDa is a multi-functional protein, binds the viral RNA to form the helical core structure as well helping in signal transduction and viral packaging (Hiscox *et al*., 2001).Matrix membrane protein of 25 kDa is the most abundantly produced and is essential protein for assembly of both enveloped and naked viral particles (Kuo and Masters, 2002) .

In the course of virion production the nucleocapsid protein dimerizes,a process which leads to capsid formation. Studies from yeast two hybrid system suggest that the stretch of 209 amino-acids helix rich region of the C terminal is crucial for this protein-protein interaction (Surjit *et al*., 2004).

It has been established that SARS-CoVnucleocapsid (N) and the membrane (M) proteins interact. Mammalian two hybrid system reveals that amino acid residues from 168-208 in the N protein were found to be important in the maintenance of precise conformation of the protein along with its M protein interaction (He *et al*., 2004).

The envelope protein E of the SARS-CoV is a small membrane protein of 76 amino acids. Its role in viral pathogenesis was initially unknown but it is now reported to be a virulence factor (DeDiego *et al*., 2008). Envelope

**Table 3.** List of influenza A virus protein-protein interactions.

| | Viral Protein 1 | | | Viral Protein 2 | | |
|---|---|---|---|---|---|---|
| **S#** | **Virus Type** | **NCBI Reference Sequence** | **Protein** | **Virus Type** | **NCBI Reference Sequence** | **Protein** |
| 1. | H3N2 | ABB04933 | nonstructural protein 1 | H3N2 | ABB04936 | polymerase PB1 |
| 2. | H3N2 | ABB04935 | polymerase PA | H3N2 | ABB04936 | polymerase PB1 |
| 3. | H3N2 | ABB04936 | polymerase PB1 | H3N2 | ABB04938 | polymerase PB2 |
| 4. | H3N2 | ABB04933 | nonstructural protein 1 | H3N2 | ABB04938 | polymerase PB2 |
| 5. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040986 | polymerase PA |
| 6. | H1N1 | NP_040985 | polymerase 1 | H1N1 | NP_040986 | polymerase PA |
| 7. | H1N1 | NP_040985 | polymerase 1 | H1N1 | NP_040987 | PB2 protein |
| 8. | H1N1 | NP_040980 | haemagglutinin | H1N1 | NP_040986 | polymerase PA |
| 9. | H1N1 | NP_040980 | haemagglutinin | H1N1 | NP_040985 | polymerase 1 |
| 10. | H1N1 | NP_040980 | haemagglutinin | H1N1 | NP_040982 | nucleocapsid protein |
| 11. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040982 | nucleocapsid protein |
| 12. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040981 | neuraminidase |
| 13. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040978 | matrix protein 1 |
| 14. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040983 | nonstructural protein NS2 |
| 15. | H1N1 | NP_040981 | neuraminidase | H1N1 | NP_040985 | polymerase 1 |
| 16. | H1N1 | NP_040982 | nucleocapsid protein | H1N1 | NP_040987 | PB2 protein |
| 17. | H1N1 | NP_040982 | nucleocapsid protein | H1N1 | NP_040982 | nucleocapsid protein |
| 18. | H1N1 | NP_040984 | nonstructural protein NS1 | H1N1 | NP_040987 | PB2 protein |
| 19. | H1N1 | NP_040984 | nonstructural protein NS1 | H1N1 | NP_040984 | nonstructural protein NS1 |
| 20. | H1N1 | NP_040984 | nonstructural protein NS1 | H1N1 | NP_040986 | polymerase PA |
| 21. | H1N1 | NP_040983 | nonstructural protein NS2 | H1N1 | NP_040984 | nonstructural protein NS1 |
| 22. | H1N1 | NP_040984 | nonstructural protein NS1 | H1N1 | NP_040985 | polymerase 1 |
| 23. | H1N1 | NP_040981 | neuraminidase | H1N1 | NP_040984 | nonstructural protein NS1 |
| 24. | H1N1 | NP_040982 | nucleocapsidprotein | H1N1 | NP_040984 | nonstructuralprotein NS1 |
| 25. | H1N1 | NP_040980 | haemagglutinin | H1N1 | NP_040984 | nonstructural protein NS1 |
| 26. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040984 | nonstructural protein NS1 |
| 27. | H1N1 | NP_040983 | nonstructural protein NS2 | H1N1 | NP_040987 | PB2 protein |
| 28. | H1N1 | NP_040982 | nucleocapsid protein | H1N1 | NP_040986 | polymerase PA |

| | | | | | | |
|---|---|---|---|---|---|---|
| 29. | H1N1 | NP_040986 | polymerase PA | H1N1 | NP_040987 | PB2 protein |
| 30. | H1N1 | NP_040986 | polymerase PA | H1N1 | NP_040986 | polymerase PA |
| 31. | H1N1 | NP_040981 | neuraminidase | H1N1 | NP_040986 | polymerase PA |
| 32. | H1N1 | NP_040983 | nonstructural protein NS2 | H1N1 | NP_040986 | polymerase PA |
| 33. | H1N1 | NP_040978 | matrix protein 1 | H1N1 | NP_040985 | polymerase 1 |
| 34. | H1N1 | NP_040985 | polymerase 1 | H1N1 | NP_040985 | polymerase 1 |
| 35. | H1N1 | NP_040983 | nonstructural protein NS2 | H1N1 | NP_040985 | polymerase 1 |
| 36. | H1N1 | NP_040982 | nucleocapsid protein | H1N1 | NP_040985 | polymerase 1 |
| 37. | H1N1 | NP_040987 | PB2 protein | H1N1 | NP_040987 | PB2 protein |
| 38. | H1N1 | ABF47959 | nucleocapsid protein | H1N1 | ABF47963 | polymerase PB1 |
| 39. | H1N1 | ABF47962 | polymerase PA | H1N1 | ABF47963 | polymerase PB1 |
| 40. | H1N1 | ABF47963 | polymerase PB1 | H1N1 | ABF47964 | PB1-F2 protein |

protein is ubiquinated by the NS3 protein which is involved in the replicase complex of virus and this binding is mediated by the N-terminal ubiquitin-like domain-1 of NS3. This interaction was proved using a system of recombinant SARS-CoV expressing tagged E protein (Álvarez *et al*., 2010). Ubiquination of the E protein plays a crucial role in the multistep life cycle of the virus especially for viral entry into host cell as its inhibition leads not only to impaired entry but also RNA synthesis. (Raaben *et al*., 2010)

It is evident from above studies that the assembly of SARS-CoV is a multistep process and it involves protein-RNA and protein-protein interactions. Identification of residues involved in this type of interactions can prove to be pivotal as potential drug targets to inflect the SARS-CoV infections.

SARS coronavirus 3C like proteinase is crucial for the maturity of the viral particle. Dimerization of the proteinase is important for its activity. The dimerization reaction may be substrate–induced that renders the proteinase active (Li *et al*., 2010). The monomeric form of SARS coronavirus 3C like proteinase is not proteolytically active (Okamoto *et al*., 2010). The residue most important for SARS coronavirus dimerization is Asn28 (Barrila *et al*., 2010). Complete inactivation of the enzyme takes place by the mutation of Asn28 to alanine. Dimerization is driven by the key amino acid residues ensuring long range interaction in spite of direct interaction at the dimerization interface. The key residues involved are Ser147, Ser144 and Cys117. An extensive network of hydrogen bonds surrounds the active site of the protein. The interaction between Ser147 and Ser144 is important in the correct positioning of Met6. Met6 plays a vital role in SARS coronavirus dimerization (Barrila *et al*., 2010). The key residue Gly-11 has also been reported to be crucial for dimerization. Mutation in Gly-11 results in disruption of SARS coronavirus dimerization. The disruption results by the shortening of the alpha-helix of domain1. Moreover, the N-terminal finger cannot hold tight into the pocket of another monomer due to its dis-orientation (Chen *et al*., 2008). SARS coronavirus 3C proteinase cleaves at conserved proteolytic cleavage site containing residues GLn85 and Leu64 of NS7. These residues are crucial for the proteolytic cleavage by the 3C proteinase (Peti *et al*., 2005).

NS7 and NS8 belong to larger hetero-multimers(Su *et al*., 2006). NS7 and NS8 interact and form a hexadecamer. This resulting haxadecamer super complex can bind dsRNA. The property of binding dsRNA is conferred to the hexadecamer by the formation of a central channel. This central channel has properties that favour electrostatic interaction with nucleic acid (Zhai *et al*., 2005).

SARS coronavirus NS8 interacts with itself forming a multimeric complex (Su *et al*., 2006). NS9 has been reported to co-localize with the nucleocapsid protein and the 3C like proteinase along with NS7 and NS8 in the late endosome. The late endosomes are the sites for SARS coronavirus genome replication (Miknis *et al*., 2009).

(Imbert *et al*., 2008; Kumar *et al*., 2007; Luo *et al*., 2005; Pan *et al*., 2008; Surjit *et al*., 2004; von Brunn *et al*., 2007) have studied genome wide protein-protein interactions of SARS corona virus listed in Table 4.

## Conclusion

There are many biochemical and physical assays which are in use for the determination of protein-protein interactions e.g. immunoprecipitation, protein affinity chromatography,

**Table 4.** List of SARS corona virus Protein-Protein interaction.

| | Viral Protein 1 | | Viral Protein 2 | |
|---|---|---|---|---|
| **S#** | **NCBI Reference Sequence** | **Protein** | **NCBI Reference Sequence** | **Protein** |
| 1 | NP_828855 | matrix protein | NP_828858 | nucleocapsid protein |
| 2 | NP_828856 | hypothetical protein sars6 | NP_828866 | nsp8-pp1a/pp1ab |
| 3 | NP_828858 | nucleocapsid protein | NP_828858 | nucleocapsid protein |
| 4 | NP_828866 | nsp8-pp1a/pp1ab | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 5 | NP_828869 | rna-dependent rna polymerase | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 6 | NP_828870 | nsp13-pp1ab (zd, ntpase/hel | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 7 | NP_828859 | hypothetical protein sars9b | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 8 | NP_828866 | nsp8-pp1a/pp1ab | NP_828871 | 3-to-5 exonuclease |
| 9 | NP_828859 | hypothetical protein sars9b | NP_828871 | 3-to-5 exonuclease |
| 10 | NP_828859 | hypothetical protein sars9b | NP_828872 | endoRNAse |
| 11 | NP_828854 | protein e | NP_828860 | leader protein |
| 12 | NP_828854 | protein e | NP_828866 | nsp8-pp1a/pp1ab |
| 13 | NP_828854 | protein e | NP_904321 | nsp11-pp1a |
| 14 | NP_828854 | protein e | NP_828854 | protein E |
| 15 | NP_828852 | hypothetical protein sars3a | NP_828860 | leader protein |
| 16 | NP_828861 | counterpart of mhv p65 | NP_828861 | counterpart of MHV p65 |
| 17 | NP_828861 | counterpart of mhv p65 | NP_904322 | nsp4-pp1a/pp1ab |
| 18 | NP_828861 | counterpart of mhv p65 | NP_828864 | nsp6-pp1a/pp1ab (TM3) |
| 19 | NP_828861 | counterpart of mhv p65 | NP_828866 | nsp8-pp1a/pp1ab |
| 20 | NP_828861 | counterpart of mhv p65 | NP_904321 | nsp11-pp1a |
| 21 | NP_828861 | counterpart of mhv p65 | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 22 | NP_828852 | hypothetical protein sars3a | NP_828861 | counterpart of MHV p65 |
| 23 | NP_828861 | counterpart of mhv p65 | NP_828862 | nsp3-pp1a/pp1ab |
| 24 | NP_828852 | hypothetical protein sars3a | NP_828862 | nsp3-pp1a/pp1ab |
| 25 | NP_828859 | hypothetical protein sars9b | NP_828862 | nsp3-pp1a/pp1ab |
| 26 | NP_828863 | 3c-like proteinase | NP_828863 | 3C-like proteinase |
| 27 | NP_828863 | 3c-like proteinase | NP_828865 | nsp7-pp1a/pp1ab |
| 28 | NP_828863 | 3c-like proteinase | NP_828866 | nsp8-pp1a/pp1ab |
| 29 | NP_828859 | hypothetical protein sars9b | NP_828863 | 3C-like proteinase |

| | | | | |
|---|---|---|---|---|
| 30 | NP_828864 | nsp6-pp1a/pp1ab (tm3) | NP_828866 | nsp8-pp1a/pp1ab |
| 31 | NP_828865 | nsp7-pp1a/pp1ab | NP_828865 | nsp7-pp1a/pp1ab |
| 32 | NP_828865 | nsp7-pp1a/pp1ab | NP_828866 | nsp8-pp1a/pp1ab |
| 33 | NP_828859 | hypothetical protein sars9b | NP_828865 | nsp7-pp1a/pp1ab |
| 34 | NP_828866 | nsp8-pp1a/pp1ab | NP_828866 | nsp8-pp1a/pp1ab |
| 35 | NP_828859 | hypothetical protein sars9b | NP_828866 | nsp8-pp1a/pp1ab |
| 36 | NP_828864 | nsp6-pp1a/pp1ab (tm3) | NP_828867 | nsp9-pp1a/pp1ab |
| 37 | NP_828865 | nsp7-pp1a/pp1ab | NP_828867 | nsp9-pp1a/pp1ab |
| 38 | NP_828866 | nsp8-pp1a/pp1ab | NP_828867 | nsp9-pp1a/pp1ab |
| 39 | NP_828852 | hypothetical protein sars3a | NP_828868 | formerly known as growth-factor-like protein (GFL) |
| 40 | NP_828859 | hypothetical protein sars9b | NP_828868 | formerly known as growth-factor-like protein (GFL) |
| 41 | NP_828866 | nsp8-pp1a/pp1ab | NP_828869 | RNA-dependent RNA polymerase |
| 42 | NP_828852 | hypothetical protein sars3a | NP_828869 | RNA-dependent RNA polymerase |
| 43 | NP_828859 | hypothetical protein sars9b | NP_828869 | RNA-dependent RNA polymerase |
| 44 | NP_828865 | nsp7-pp1a/pp1ab | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 45 | NP_828853 | hypothetical protein sars3b | NP_828854 | protein E |
| 46 | NP_828854 | protein e | NP_849175 | hypothetical protein sars7b |
| 47 | NP_828854 | protein e | NP_828859 | hypothetical protein sars9b |
| 48 | NP_828852 | hypothetical protein sars3a | NP_828855 | matrix protein |
| 49 | NP_828852 | hypothetical protein sars3a | NP_828858 | nucleocapsid protein |
| 50 | NP_828856 | hypothetical protein sars6 | NP_828862 | nsp3-pp1a/pp1ab |
| 51 | NP_828856 | hypothetical protein sars6 | NP_849175 | hypothetical protein sars7b |
| 52 | NP_828857 | hypothetical protein sars7a | NP_828862 | nsp3-pp1a/pp1ab |
| 53 | NP_828851 | e2 glycoprotein precursor | NP_828857 | hypothetical protein sars7a |
| 54 | NP_849176 | hypothetical protein sars8a | NP_828866 | nsp8-pp1a/pp1ab |
| 55 | NP_849176 | hypothetical protein sars8a | NP_828872 | endoRNAse |
| 56 | NP_849176 | hypothetical protein sars8a | NP_849177 | hypothetical protein sars8b |
| 57 | NP_828859 | hypothetical protein sars9b | NP_849176 | hypothetical protein sars8a |
| 58 | NP_849177 | hypothetical protein sars8b | NP_828862 | nsp3-pp1a/pp1ab |
| 59 | NP_849177 | hypothetical protein sars8b | NP_828866 | nsp8-pp1a/pp1ab |

| | | | | |
|---|---|---|---|---|
| 60 | NP_828851 | e2 glycoprotein precursor | NP_849177 | hypothetical protein sars8b |
| 61 | NP_849175 | hypothetical protein sars7b | NP_849177 | hypothetical protein sars8b |
| 62 | NP_828859 | hypothetical protein sars9b | NP_849177 | hypothetical protein sars8b |
| 63 | NP_828859 | hypothetical protein sars9b | NP_849175 | hypothetical protein sars7b |
| 64 | NP_828859 | hypothetical protein sars9b | NP_828859 | hypothetical protein sars9b |
| 65 | NP_828862 | nsp3-pp1a/pp1ab | NP_828862 | nsp3-pp1a/pp1ab |
| 66 | NP_828862 | nsp3-pp1a/pp1ab | NP_904322 | nsp4-pp1a/pp1ab |
| 67 | NP_828862 | nsp3-pp1a/pp1ab | NP_828869 | RNA-dependent RNA polymerase |
| 68 | NP_828862 | nsp3-pp1a/pp1ab | NP_828866 | nsp8-pp1a/pp1ab |
| 69 | NP_828861 | counterpart of mhv p65 | NP_828865 | nsp7-pp1a/pp1ab |
| 70 | NP_828853 | hypothetical protein sars3b | NP_828866 | nsp8-pp1a/pp1ab |
| 71 | NP_849175 | hypothetical protein sars7b | NP_828867 | nsp9-pp1a/pp1ab |
| 72 | NP_828868 | formerly known as growth-factor-like protein (gfl) | NP_828871 | 3-to-5 exonuclease |
| 73 | NP_828868 | formerly known as growth-factor-like protein (gfl) | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 74 | NP_828863 | 3c-like proteinase | NP_828869 | RNA-dependent RNA polymerase |
| 75 | NP_828853 | hypothetical protein sars3b | NP_828869 | RNA-dependent RNA polymerase |
| 76 | NP_828853 | hypothetical protein sars3b | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 77 | NP_828863 | 3c-like proteinase | NP_828871 | 3-to-5 exonuclease |
| 78 | NP_828853 | hypothetical protein sars3b | NP_828871 | 3-to-5 exonuclease |
| 79 | NP_828861 | counterpart of mhv p65 | NP_828872 | endoRNAse |
| 80 | NP_828872 | endornase | NP_828872 | endoRNAse |
| 81 | NP_849175 | hypothetical protein sars7b | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 82 | NP_828858 | nucleocapsid protein | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 83 | NP_828852 | hypothetical protein sars3a | NP_849177 | hypothetical protein sars8b |
| 84 | NP_828854 | protein e | NP_828857 | hypothetical protein sars7a |
| 85 | NP_828855 | matrix protein | NP_828857 | hypothetical protein sars7a |
| 86 | NP_828855 | matrix protein | NP_849177 | hypothetical protein sars8b |
| 87 | NP_849177 | hypothetical protein sars8b | NP_828867 | nsp9-pp1a/pp1ab |
| 88 | NP_828858 | nucleocapsid protein | NP_849177 | hypothetical protein sars8b |
| 89 | NP_828862 | nsp3-pp1a/pp1ab | NP_828863 | 3C-like proteinase |
| 90 | NP_828862 | nsp3-pp1a/pp1ab | NP_828870 | nsp13-pp1ab (ZD, |

| | | | | |
|---|---|---|---|---|
| | | | | NTPase/HEL |
| 91 | NP_828862 | nsp3-pp1a/pp1ab | NP_828871 | 3-to-5 exonuclease |
| 92 | NP_828862 | nsp3-pp1a/pp1ab | NP_828864 | nsp6-pp1a/pp1ab (TM3) |
| 93 | NP_828865 | nsp7-pp1a/pp1ab | NP_828869 | RNA-dependent RNA polymerase |
| 94 | NP_828865 | nsp7-pp1a/pp1ab | NP_828871 | 3-to-5 exonuclease |
| 95 | NP_828866 | nsp8-pp1a/pp1ab | NP_828872 | endoRNAse |
| 96 | NP_828860 | leader protein | NP_828867 | nsp9-pp1a/pp1ab |
| 97 | NP_828867 | nsp9-pp1a/pp1ab | NP_828871 | 3-to-5 exonuclease |
| 98 | NP_828863 | 3c-like proteinase | NP_828868 | formerly known as growth-factor-like protein (GFL) |
| 99 | NP_828862 | nsp3-pp1a/pp1ab | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 100 | NP_828861 | counterpart of mhv p65 | NP_828869 | RNA-dependent RNA polymerase |
| 101 | NP_828869 | rna-dependent rna polymerase | NP_828871 | 3-to-5 exonuclease |
| 102 | NP_828869 | rna-dependent rna polymerase | NP_828872 | endoRNAse |
| 103 | NP_828869 | rna-dependent rna polymerase | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 104 | NP_828866 | nsp8-pp1a/pp1ab | NP_828868 | formerly known as growth-factor-like protein (GFL) |
| 105 | NP_828866 | nsp8-pp1a/pp1ab | NP_828873 | 2-O-ribose methyltransferase (2-o-MT) |
| 106 | NP_828862 | nsp3-pp1a/pp1ab | NP_828865 | nsp7-pp1a/pp1ab |
| 107 | NP_828862 | nsp3-pp1a/pp1ab | NP_828872 | endoRNAse |
| 108 | NP_828861 | counterpart of mhv p65 | NP_828867 | nsp9-pp1a/pp1ab |
| 109 | NP_828862 | nsp3-pp1a/pp1ab | NP_828867 | nsp9-pp1a/pp1ab |
| 110 | NP_828863 | 3c-like proteinase | NP_828867 | nsp9-pp1a/pp1ab |
| 111 | NP_828867 | nsp9-pp1a/pp1ab | NP_828868 | formerly known as growth-factor-like protein (GFL) |
| 112 | NP_828867 | nsp9-pp1a/pp1ab | NP_828869 | RNA-dependent RNA polymerase |
| 113 | NP_828867 | nsp9-pp1a/pp1ab | NP_828870 | nsp13-pp1ab (ZD, NTPase/HEL |
| 114 | NP_828867 | nsp9-pp1a/pp1ab | NP_828872 | endoRNAse |

affinity blotting and western blot experiments. Recent advances in high-throughput methods for the detection of protein-protein interaction, such as yeast two-hybrid and mass spectrometry techniques, have led to a rapid expansion of such data for a wide range of organisms. In such a way, several studies address the viral-viral and viral-host protein interactions depicting the molecular mechanism involved in the pathogenicity of viruses. Most of the pathogenicity occurs due to the interaction of host and viral molecular factors which ultimately affect normal cellular signaling pathways. But the first line of viral action is viral protein-protein interactions which on the second hand initiate their pathogenesis. Anti-viral therapies based on blocking viral-host interactions come with certain discrepancies such as disturbances of the normal cellular processes. New treatment options specific in targeting viral protein-protein interaction can be proved fruitful. At this time, new classes of inhibitors are needed which specifically hinder only the post modifications of viral proteins by other virus encoded proteins. This treatment strategy will definitely prove to be a better option with safe hands on host molecular identities.

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