SUMMARY

Host-Cell Actin Cytoskeleton Interactions With Viral Proteins During Viral Infection.

Cytoskeleton is a major intracellular structure in eukaryotic cells. It is a complex organization which performs a variety of functions, including maintenance of the cell shape and integrity during stress or intracellular infections. It mainly consists of microfilaments, intermediate filaments and microtubules. Of these, actin containing microfilaments (often referred to as "actin cytoskeleton") are critical for many aspects of the cell behaviour. In addition to maintaining the cell morphology, it is required for cell motility, cell division and intracellular transport. The second of the cytoskeletal elements, intermediate filaments (IFs), are the major fibrous proteins that are found in the nucleoplasm and the cytoplasm of the most types of animal cells. In the cytoplasm they are usually organized in the perinuclear region, where they frequently form a cage that surrounds and appears to position in the nucleus. IFs link the nuclear and cell surfaces and are involved in numerous cell functions, including maintenance of overall integrity of cytoplasm and the cell shape. The third element of cytoskeleton, the microtubules, are ubiquitous cytoskeletal structures that are formed by the self assembly of αβ tubulin heterodimers. Microtubules are involved in diverse functions that include cell movement, vesicle transport and chromosome segregation during mitosis.

During intracellular infections, integrity and organization of cytoskeletal components are often altered. Of the three filaments, actin filaments are the major target of pathogens including viruses. Numerous viruses interact with actin at various stages throughout their life cycle, both disrupting and rearranging the actin cytoskeleton to their own advantage. The most spectacular example is the vaccinia virus, which not only disrupts the actin cytoskeleton at an early stage of infection but at a later stage it also uses actin polymerization to propel itself into neighboring cells.

One or more proteins of many viruses interact with the actin filaments in the virus-infected cells. Some of the viral proteins have also been shown to interact with actin *in vitro*. It is now becoming increasingly clear that viruses use actin

cytoskeleton for their propagation inside the cells. Based on several reports describing the interactions of viral proteins with cytoskeletal actin as well as on the existing knowledge on cellular functions of cell cytoskeleton, it is proposed that the intracellular virus modifies the host cell cytoskeleton by putting one or more of its proteins into the cytoskeletal apparatus to facilitate its growth and propagation. The work embodied in the thesis was undertaken to analyse the interaction of influenza viral protein with actin cytoskeleton and then the effect of this interaction on virus budding.

Interaction of Influenza Viral Nucleoprotein (NP) and Matrix protein (MP) with Actin

Influenza virus is an externally enveloped virus which buds out from the plasma membrane of the host cell. NP is a major structural protein of influenza virus particle, which performs multiple functions during the viral infection. MP, on the other hand , is the most abundant protein in the virion which forms a shell beneath the lipid bilayer of the virus particle. Both NP and MP coextracted with the cytoskeleton of the virus-infected MDCK cells. During extraction of the infected cell cytoskeleton in the presence of salts or upon the treatment of infected cell cytoskeleton with buffers containing high concentrations of various salts, there was considerable release of these proteins from the cytoskeleton. This was interpreted to suggest that the nature of interactions between the viral proteins and cytoskeleton was predominantly ionic and that these proteins mainly interact with actin. During intracellular localization of NP and MP in virus-infected cells by double immunofluorescence and confocal microscopy, these proteins were found to closely associated with actin filaments. Upon treatment of the virus-infected cells with the microfilaments-disrupting drug cytochalasin(s), both these viral proteins were seen to colocalize with the actin filaments, especially on membrane extensions called as blebs. On the other hand, hardly any colocalization of NP and MP was observed with the other two components of the cytoskeleton, viz. IFs and microtubules, by double immunofluorescence or confocal microscopy. Based on

these findings, we conclude that both NP and MP of influenza virus interact with actin filaments of the cytoskeleton.

Both N- and C-Termini of NP are Involved in Interaction with Cytoskeleton

During the progress of this work one report has suggested that NP interacts directly with the cytoskeleton while MP-cytoskeleton interactions seems to be indirect, probably mediated by some other protein or factor. To further confirm the NP-actin cytoskeleton interactions, influenza viral NP was expressed in COS1 cells. Both NP and actin could be coimmunoprecipitated using anti-NP and anti-actin antibodies, which confirmed the NP-cytoskeleton interaction. To identify the region(s) or domain(s) of NP involved in these interactions, a series of deletion mutants were constructed and expressed in COS1 cells. The cytoskeletons of the cells expressing the mutants were extracted and their interaction with truncated NPs was studied. The immunoprecipitation analysis, suggested both N-and C-termini of NP are involved in its interaction with the cytoskeleton.

Both NP and MP play a major role in virus assembly as well as in virus budding. These two proteins interact with each other in the nucleus and also in the cytoplasm. MP is required for exporting the viral RNPs out of the nucleus and for preventing the re-entry of the exported out viral RNPs back to the nucleus. NP, on the other hand, binds to the viral RNA in the nucleus to form the viral RNP which traverse to the budding sites at the membrane predecorated with viral glycoproteins, viz. HA and NA, to affect the final virus assembly and subsequent budding. This process in principle should require an active involvement of the host cell cytoskeleton apparatus, as the various components of cytoskeleton are known to be required for guiding and movement of the intracellular material to the plasma membrane. In addition, cytoskeleton also required for modifying the plasma membrane particle distribution and deformability properties. After entering the nucleus MP forms a complex with viral RNPs through its association with NP and then this complex migrates to the cytosol where it binds the cytoskeleton through MP to prevent its re-entry back into the nucleus. Alternatively, NP may associate

vith MP in the cytosol to induce a binding between MP and cytoskeletal actin, vhich inturn could serve as the capture sites for viral RNPs being exported out rom the nucleus. It would therefore seem that the binding between the host cell ytoskeletal actin and the viral proteins may prove useful to virus by providing to it vith a mechanism which can prevent the re-entry of viral RNPs back into the ucleus.

Binding of MP with the cytoskeleton seems to be indirect and probably nediated by NP. NP interact with cytoskeleton directly and its both N- and C- ermini involved in this binding. MP is also believed to decorate the cytoplasmic ace of the plasma membrane which serves as the site for virus budding and subsequently as the viral membrane. Here we propose that at the virus budding ite MP interacts with viral RNP which is already associated with the actin ytoskeleton through NP. This complex should, in principle, modify the ytoskeleton structure by affecting the intermolecular interactions within the ytoskeletal matrix, which could, inturn, facilitate its dissociation from the overlying membrane bilayer and allow subsequent budding of the virus.