Groundnut Bud Necrosis Virus (GBNV) is a tripartite ambisense RNA plant virus that belongs to serogroup IV of Tospovirus genus. Non-Structural protein-m (NSm), which functions as movement protein in tospoviruses, is encoded by the M RNA. In this chapter, we demonstrate that despite the absence of any putative transmembrane domain, GBNV NSm associates with membranes when expressed in *E. coli* as well as in *N. benthamiana*. Incubation of refolded NSm with liposomes ranging in size from 200-250 nm resulted in changes in the secondary and tertiary structure of NSm. A similar behaviour was observed in the presence of anionic and zwitterionic detergents. Furthermore, the morphology of the liposomes was found to be modified in the presence of NSm. Deletion of coiled coil domain resulted in the inability of *in planta* expressed NSm to interact with membranes. Further, when the C-terminal coiled coil domain alone was expressed, it was found to be associated with membrane. These results demonstrate that NSm associates with membranes via the C-terminal coiled coil domain and such an association may be important for movement of viral RNA from cell to cell. Further NSm was shown to be phosphorylated by *N. benthamiana* and tomato crude sap as observed in other movement proteins.

This chapter deals with localization of NSm to PD and identification of domain involved in localization. For this purpose NSm and its mutants were cloned in pEAQ:GFP vector and transiently expressed in N. benthamiana by infiltration of transformed Agrobacteria. The GFP tagged NSm was visualized by confocal microscopy. The results demonstrated that NSm forms punctate structures and localizes to PD as confirmed by colocalization of mCherry: PDLP1a, a PD marker which resides in PD, with GFP:NSm. To find out the domain involved in PD localization, sequential deletion mutants were made. It was found that C-terminal domain is involved in PD localization. On the other hand, N-terminal unfolded region was dispensable for PD localization. This is the first report of a coiled coil domain shown to be involved in PD localization. It has also been demonstrated that GBNV NSm interacts with NP. Further, membrane floatation assay carried in presence of NP suggested that interaction of NSm and NP affected membrane association of NSm. These results were further confirmed by localization studies of NSm in presence of NP. It was found that there was considerable relocalization of both NSm and NP. NSm was observed to be present in cytoplasm as well as on the membrane. At the same time, NP was observed on membrane apart from being present in the cytoplasm. When N- terminal 50 amino acids (unfolded) region of NSm was deleted and colocalization studies were carried out, it was found that NSm and NP do not colocalize, suggesting that NSm interacts with NP via the unfolded region and helps in the relocalization of NP to the membrane.

This chapter deals with the pathway of targeting NSm to PD. To decipher the pathway, followed by NSm, an inhibitor of endomembrane or vesicle mediated transport, Brefeldin A (BFA) was used. When GFP-NSm was expressed it was observed to form punctate structure at PD as before. Upon treatment with BFA, green islands were observed in the cytoplasm suggesting that ER was involved in targeting NSm to PD. Similarly, LatB, inhibitor of actin mediated targeting of protein to membrane, also abrogated the localization of NSm to PD. In order to further understand the role of ER in targeting NSm to PD, an ER marker, ER-GFP (GFP fused to HDEL peptide that directs it to ER) was coexpressed with GBNV NSm fused to mCherry. It was observed that NSm colocalizes with ER-GFP as yellow puncta on PD. The puncta appeared as patches and the whole ER-network was converted to vesicles. This was further confirmed by coexpressing ER-GFP with NSm without any tag. The green fluorescent vesicles were observed preferentially near cell membrane. To delineate the region of NSm involved in vesicle formation, point mutants and deletion mutants of NSm were generated without the tag and coexpressed with ER-GFP. When N-terminal 203 amino acids were deleted, NSm was able to transform ER membranes to vesicles suggesting that these residues are dispensable for vesicle formation. Interestingly, the deletion of coiled coil domain leads to cytosolic location of NSm. Furthermore, the C-terminal coiled coil domain when expressed alone was capable of inducing vesicle formation. This is the first report of involvement of such a domain in ER membrane association and vesicle formation.