Summary

The subcellular compartmentalization of eukaryotic cells provided them with the efficiency of segregating various cellular processes, thereby allowing them to perform discrete biological functions and evolve as numerous morphologically and functionally distinct cell types regulating physiology of human body. This level of compartmentalization was made possible because of an exclusive array of endomembranous compartments, though distinct from each other yet connected by the flow of material is regulated by a series of regulatory proteins. The defects in these regulatory proteins create an imbalance in cellular homeostasis, leading to various diseases. Small GTPases belonging to RAS superfamily constitute one of the many classes of these regulatory proteins. The role of ARF/ARL-family proteins (from RAS superfamily), Arl8b and Arl11, in the regulation of membrane trafficking and signal transduction have been investigated in this thesis work.

Chapter 2 of the thesis provides insight into the role of Arl11 in regulating innate immunity in response to infection. Although the role Arl11 as a tumor suppressor protein has been reported earlier along with the fact that it is not expressed in cancer tissue and cancer cell lines; making it pretty difficult for researchers to understand its role in the regulation of normal cell physiology. However, the knowledge that Arl11 is expressed in immune-related organs offered us an opportunity to investigate the physiological function(s) of Arl11 in immune-related tissue. Here, we report the novel role of Arl11 in regulating LPS-induced p-ERK1/2-mediated activation of macrophages. Also, the presence of Arl11 is important for phagocytosis and clearance of bacteria by macrophages. In Chapter 3 of this thesis, we have highlighted the role of sustained ERK1/2 activation in causing apoptosis of tumor cells upon ectopic expression of Arl11. Further, we provide evidence that Arl11 interacts with phosphorylated form of ERK1/2, and colocalizes with it at the cortical actin structures. At this time, we don't know the protein partners that might play a role in mediating Arl11-ERK1/2 interaction. One such plausible candidate can be IQGAP1, a scaffold protein that has been previously shown to act in actin remodeling, ERK1/2 signaling, Akt signaling components and play important role in many cellular processes such as cell migration, cell adhesion, invasion of cancer cells and regulation of endocytic trafficking at the plasma membrane (Roy *et al.*, 2004b; Mataraza *et al.*, 2003; Watanabe *et al.*, 2004; Samson *et al.*, 2017). Based on these evidences, we hypothesize that Arl11 at cortical actin structures might aid recruitment of IQGAP1 over cortical actin, making it more accessible to growth, migration or

phagocytic signals thereby transmitting them to ERK1/2 or other signal transduction molecules. The presence of RAS-GAP-like domain in IQGAP1 raises the possibility of its binding to Arl11 which needs to be tested in future studies. Also, Arl11, like other small GTPases, might be regulating its functions based on GTP/GDP bound state, therefore the identification GEF, GAP, and effectors of Arl11 will help in uncovering new roles of Arl11.

In our studies presented in Chapter 3 of this thesis, we report nucleo-cytoplasmic distribution of Arl11. Although presently we were unable to address the role of this localization in regulating cellular functions, future studies in this direction will greatly help in better understanding the contribution of nuclear versus cytosolic pool of Arl11 in mediating macrophage activation and/or its tumor suppressor function.

In Chapter 4, we have presented our published work related to identification of PLEKHM1 as a novel effector of Arl8b. We also showed that PLEKHM1 is a shared effector of both Rab7 and Arl8b, linking them together thereby helping in autophagosome/endosome-lysosome fusion (Marwaha *et al.*, 2017). Our finding that both PLEKHM1 and SKIP, the effectors of Arl8b, compete with each other for binding to Arl8b and thereby regulate lysosomes positioning in the cells. Mutations in *PLEKHM1* that causes impairment of lysosome acidification and increased bone absorption leading to aggravation of osteoporosis has been reported in a recent study (Van Wesenbeeck *et al.*, 2007; Del Fattore *et al.*, 2008; Fujiwara *et al.*, 2016). As regulation of lysosomal positioning is at the center of cellular processes in many cell types such as neurons, polarized epithelial cells, and osteoclasts, and Arl8b being an important modulator of lysosomal positioning to date; further studies are needed to reveal novel effectors of Arl8b that might perform cell type specific functions.

Overall, the work presented in this thesis highlights the diverse functions performed by Arf-like (Arl) GTPases (in particular by Arl11 and Arl8b) in regulating membrane trafficking and maintaining cellular homeostasis.