Summary
The intracellular pathogen *Salmonella*, during its course of infection, invades the intestinal epithelial cells and macrophages of the spleen and liver, where the bacterium resides and replicates (Richter-Dahlfors et al., 1997). Survival within macrophages is an essential part of *Salmonella* pathogenesis since mutants lacking this ability have been shown to be avirulent (Fields et al., 1986). Studies over the years have led to the conclusion that *Salmonella* survives in an intracellular niche inside macrophages by inhibiting its transport to the lysosomes (Buchmeier and Heffron, 1991). Though, several groups have tried to elucidate the mechanism of inhibition of transport, it has still not been fully comprehended. However, a general consensus has developed on the role of certain effector molecules secreted by *Salmonella* that modulate the host cellular processes, accomplishing pathogen survival in macrophages.

Previous studies from our lab have identified one such *Salmonella* effector protein, SopE. It has been shown to recruit one of the regulators of host intracellular transport, Rab5, onto the phagosomal membrane and promote fusion of the phagosomes with early endosomes, subverting the compartment from being targeted to lysosomes (Hashim et al., 2000; Mukherjee et al., 2000). Recent data from the lab has shown a temporal acquisition of host SNARE molecules during the maturation of *Salmonella*-containing phagosomes. Based on the premise that SNARE recruitment must have been brought about by some bacterial effectors, the present study aimed at identifying them and determining their role in bacterial survival inside macrophages.

Initially, three different molecules from a subclass of host SNARE proteins, namely, syntaxin 6, syntaxin 7 and syntaxin 8, were cloned and expressed as GST-tagged fusion proteins. Subsequently, these fusion proteins were used as baits to pull down effector molecules from *Salmonella* and characterize them. Our results demonstrate that SipC, a SPI-1 effector protein of *Salmonella*, specifically interacts with host syntaxin 6. This interaction was confirmed *in vitro* by Western blotting using recombinant proteins as well as *in vivo* by immunoprecipitation where *Salmonella* SipC could specifically pull out syntaxin 6 from macrophage lysate. Thereafter, the presence of SipC on the phagosomes implicated its role in regulating the host molecules and thus, contributing to the phagosomal maturation process. To decipher the physiological role(s) of SipC, we deleted this molecule from the *Salmonella* genome.
Interactions of *Salmonella*-containing phagosomes with various intracellular compartments were then compared between WT as well as *sipC* knockout bacteria. However, no significant differences were found; the mutant bacteria were still able to interact efficiently with members of the host endocytic pathway. This has been illustrated by the observations that *sipC* knockout *Salmonella* obtained early endosomal markers such as Rab5, followed by subsequent dissociation of these molecules and acquisition of LBPA from the late endosomes in a similar fashion as WT *Salmonella*. This clearly indicates that SipC does not modulate the endocytic pathway to the bacteria’s advantage. On the other hand, we were able to confirm that the loss of SipC did not lead to targeting of the bacteria to the lysosomes and hence, the mutants could survive efficiently within host macrophages.

Syntaxin 6 is a TGN associated SNARE molecule, involved in regulating transport at this compartment. Considering that SipC interacts specifically with host syntaxin 6, we postulated that SipC might be involved in regulating interactions of the phagosomes with intracellular compartments of the secretory pathway. Interestingly, we observed that deletion of *sipC* leads to altered intracellular trafficking and the bacterium is not targeted to a juxtanuclear Golgi localization which, under normal circumstances, serves as its replicative niche (Salcedo and Holden, 2003). To explore this further, we compared the recruitment of different TGN related transport molecules on the maturing phagosomes and observed a differential association of many of these molecules with WT and *sipC* knockout *Salmonella*-containing phagosomes by Western blotting. The mutant *Salmonella* are unable to recruit syntaxin 6 and Rab6 on to the mature phagosomes. Moreover, the recruitment of Vti1b, a syntaxin 6 fusion complex partner as well as EEA-1, a syntaxin 6 interacting molecule, was also hampered. Taken together these results confirmed the previous observation that there was no defect in interactions of *Salmonella*-containing phagosomes with members of the host endocytic machinery. On the other hand, it was the TGN associated transport molecules whose acquisition was crippled only on mature phagosomes, implicating the inability of *sipC* knockout *Salmonella*-containing phagosomes to interact efficiently with members of the secretory pathway.

Finally, we were able to restore the recruitment of host transport molecules with a *sipC* knock-in *Salmonella*. By regaining the function with a ‘knock-in’ phenotype, we have established beyond doubt that this bacterial effector protein is responsible for recruitment
of molecules from TGN. In other words, SipC mediated processes might be essential to import molecules on to the phagosomes from the secretory pathway. These observations led us to speculate that the transport of host molecules via this pathway might be hampered. So, efforts were made to monitor the transport of some host molecules from this compartment.

It has been shown that LAMP-1, after being synthesized in the ER, moves towards the early endosomal compartment and plasma membrane via TGN before being trafficked to the lysosomes (Cook et al., 2004). It has also been suggested that LAMP proteins are involved in the stabilization of the phagosomal compartment (Chakraborty et al., 1994; Roark and Haldar, 2008) and maturing phagosomes acquire LAMP-1 (Hashim et al., 2000), possibly by interaction with the secretory pathway. Hence, we chose this molecule as a marker for intracellular transport via TGN. Initial experiments examined the recruitment of LAMP-1 on maturing phagosomes containing either the WT or sipC knockout Salmonella. The results obtained show that the mutant bacteria could acquire LAMP-1 efficiently on early phagosomes. However, it failed to recruit this molecule on the mature phagosomes. This reaffirmed that SipC modulated the components of secretory pathway to help establish the bacterial intracellular niche. We validated this proposition by monitoring intravesicular fusion between LAMP-1 containing Golgi derived vesicles and Salmonella-containing phagosomes by microscopy. It turned out that whereas the two compartments could efficiently fuse in case of the WT bacteria, phagosomes containing the sipC knockout Salmonella were unable to fuse and acquire LAMP-1.

In conclusion, our results have shown that SipC is required for the trafficking of Salmonella near the Golgi in macrophages. Previously, SipC has been implicated in modulation of host actin cytoskeleton in concert with other T3SS1 effectors (Hayward and Koronakis, 1999). It has also been established that the final niche where Salmonella replicates is near Golgi and this has been attributed to the Salmonella effectors SseF, SseG and SifA. These proteins mediate the precise positioning of the phagosomes by differentially modulating the recruitment of microtubule motor proteins (Abrahams et al., 2006; Salcedo and Holden, 2003). However, in this work, we have identified another Salmonella effector, SipC, that is involved in the targeting of Salmonella-containing phagosomes towards Golgi, which might be a consequence of a similar modulation of host
cytoskeleton. Finally, we have addressed the significance of *Salmonella* homing near Golgi and shown for the first time that SipC mediated processes recruit syntaxin 6 and Rab6 to obtain LAMP-1 on *Salmonella*-containing phagosomes which might stabilize this specialized intracellular compartment in macrophages (Fig. 39).

*Figure 39: Schematic of SipC mediated subversion of host transport machinery for efficient survival of *Salmonella* within macrophages.*