Chapter 5

Conclusions and future perspectives
Work in this thesis has been directed towards studies on the processes of basic amino acid export in *E. coli*. The salient findings of this thesis are (i) in *E. coli* there appears to be a division of labor in the export of the two basic amino acids Arg and Lys, a situation that is distinct from that seen for *C. glutamicum* where a single protein LysE mediates export of both Arg and Lys, (ii) genetic and physiological studies have led to the identification and functional characterization of LysO a predicted inner membrane protein that mediates Lys export in *E. coli* and recently a protein family containing orthologs of LysO has been designated as the LysO family in Transporter Classification Database (TCDB) (http://www.tcdb.org/search/result.php?tc=2.A.124), (iii) a detailed analysis of the membrane topology of ArgO has revealed that its topology is distinct from that proposed for its ortholog LysE in that the distinction is restricted to the arrangement of the proximal one third portions of the two proteins, (iv) additional features of this study are that ArgO possesses an N\textsubscript{in}-C\textsubscript{out} configuration, bearing an indispensable cytoplasmic N-terminal domain, whose role in Arg export remains to be determined, with circumstantial evidence indicating that the functional unit for Arg export is an ArgO monomer and (v) studies that were initiated to understand the genetic basis of resistance of the *argO* mutant to Arg containing dipeptides have led to an unexpected outcome. It appears that in *E. coli* the Arg-Ala dipeptide may cause presently not well understood physiological perturbation that is mitigated by the MATE family member, YdhE and the LysE family member, ArgO, with the two proteins presumably facilitating its export.

While the data, mainly genetic, obtained on LysO are consistent with its role as a Lys exporter future experimentation will involve addressing issues related to the mechanism of LysO meditated Lys export, in particular its energetics. Experiments in this regard will involve the use of everted membrane vesicles wherein export into the lumen of labeled Lys can be detected and the influence of agents that dissipate the proton gradient, the membrane
potential or those that interfere with ATP hydrolysis, on Lys accumulation in the vesicular lumen can be assessed. Perhaps in vitro reconstitution of LysO mediated Lys export in proteoliposomes, is the most appropriate approach from a reductionist viewpoint because then one can obtain direct evidence for LysO mediated Lys export and also address issues related to the substrate selectivity. Determination of topology of LysO also constitutes a subject for future studies.

In the case of amino acid exporters it has been observed that in most instances the exported amino acid is found to influence the transcription of the exporter gene (discussed in chapter 2, section 2.4.4). However in the case of lysO expression it was surprising to find an absence of an effect of Lys on its expression (chapter 2). It may be noted that all studies on lysO expression have been performed with transcriptional fusions to lacZ thus the potential effect of Lys in mediating posttranscriptional regulation of lysO cannot be excluded at this stage. It may be therefore worthwhile to construct translational fusions of lysO to lacZ to test the probable role of Lys in mediating posttranscriptional regulation of lysO. It is tempting to imagine the existence of a Lys riboswitch modulating translation of lysO, however it may be noted that in most instances of riboswitch mediated regulation of gene expression, riboswitch regulated genes possesses a long untranslated leader RNA (Rodionov et al 2003, Serganov and Nudler 2013), which is not the case with lysO, the lysO leader is predicted to be 37 bases.

Following the delineation of the topology of ArgO, the next logical steps are to (i) delineate the mechanism of Arg/Lys export mediated by ArgO, (ii) determine the probable role of the cytoplasmic NTD (N-terminal domain) of ArgO in the Arg export mechanism and (iii) initiate efforts towards determining the structure of ArgO. While the last step constitutes a long term objective, activities related to the first two can be initiated. A bottleneck for in vitro studies of integral membrane proteins is the difficulty in obtaining sufficient quantities of pure membrane protein preparations largely owing to the toxicity associated with their
overproduction. However a valuable resource has been generated in this work which is the finding that overexpression of the Cys-less ArgO that retains ArgO function, did not lead to toxicity and thus efforts need to be directed towards obtaining sufficient quantities of Cys-less ArgO for biochemical studies. While the exact role of the ArgO NTD is as yet unclear it is speculated that the NTD may act as a mechanism to gate Arg export. If so it is worth testing whether the isolated NTD in vitro displays Arg/Lys binding and the influence of the amino acid substitutions G20C and Q22R on Arg/Lys binding can also be examined. Arg/Lys mediated structural transitions of the NTD, if at all they occur can also be delineated. As suggested for LysO, reconstitution of ArgO mediated Arg/Lys export in protepoliposomes is a requirement towards delineation of the export mechanism.

A proposition put forth in this thesis is that the Arg-Ala dipeptide causes a physiological perturbation in *E. coli* which renders an *argO ydhE* double mutant sensitive to Arg-Ala. Evidence obtained indicate that the perturbation may impact cellular amino acid pools and that the sensitivity (RA$^S$ phenotype) may not result due to Arg toxicity. The latter needs to be discounted at first and quantitation of Arg pools following transient exposure of the *argO ydhE* double mutant to both Arg-Ala and Ala-Arg and of the Arg pool of the *argO ydhE argR* triple mutant needs to be done. Furthermore quantification of the total amino acid content of the *argO ydhE* double mutant following transient exposure to Arg-Ala is expected to yield clues on the physiological defect. Perhaps adopting a metabolomics approach involving global metabolite analyses may be the way forward to define the perturbation caused by Arg-Ala in the *argO ydhE* double mutant. Lastly, it is suggested that Arg-Ala may be a proxy of a naturally occurring antimicrobial drug that is an analogue of Arg-Ala, and is an export substrate of both YdhE and ArgO. The effects of members of a large chemical library on the growth of the *argO ydhE* double mutant may aid in identification of the natural exported substrate through ArgO and YdhE.