In the present thesis we have attempted to initiate studies on the vacuolar proteolytic pathway of the fission yeast *S. pombe*. We have initiated our studies through a genetic approach and have sought to isolate mutants defective in Carboxypeptidase Y (CpY). The reason for choosing to isolate such mutants was two fold. Firstly, the isolation of similar mutants in *S. cerevesiae* led to 16 different complementation group that included mutations in genes that were required for the processing of proform of Carboxypeptidase Y to its mature form as well as vacuolar biogenesis mutants and vacuolar protein targeting mutants. Secondly , there is a simple plate assay for CpY that readily facilitates the isolation of mutants .

We isolated several mutants defective in CpY by EMS and NTG mutagenesis. The mutants were recessive. Secondary phenotype analysis of the mutants revealed that the mutants were neither ion sensitive nor temperature sensitive and unlikely therefore to be vacuolar biogenesis mutants. Complementation analysis of the mutants revealed that all the mutants fell into single complementation group *pcy*1.

We cloned the gene complementing the *pcy*1-3 mutation by transformation with an *S. pombe* genomic library. The complementing plasmid that was obtained was mapped and suitable subclones were made for checking minimum complementing fragment and to intiate its sequencing. We sequenced more than half of the gene when we discovered that the entire cosmid C19g12 containing this gene was sequenced as a part of *S. pombe* genome sequencing project at the Sanger Sequencing Center, UK. The sequence however, is yet to be deposited in the public databases. Sequence analysis of the complementing region showed an unusually large ORF which has significant homology to the C-terminal region of the Carboxypeptidase Y genes of *P. pastoris*, *S. cerevisiae* and *C. albicans*. The hydropathy plot revealed an N-terminal putative signal sequence, followed by an unusual long proline rich hydrophilic region that included repeat sequence of 11 repeats of MPPPPMHHEPGEH and 7 repeats of HHKGPKDKE.

The enzyme we had cloned was 1002 amino acids long and was twice the size of the similar Carboxypeptidase Y homologs of 525-550 amino acids length. The extra length of the *S. pombe* CpY codes for the N-terminal repeat region. However, we were unable to decipher the role of this repeat region. To determine if this gene was indeed coding for the functional CpY, we carried out a genomic disruption of the gene in *S. pombe*. Two disruptants were made, one in the N-terminal repeat region, and the second in the mature region having the catalytic site. Both the disruptants led to a loss in Carboxypeptidase Y activity, indicating that the gene encodes for a functional Carboxypeptidase Y.