Summary
SUMMARY

*Entamoeba histolytica* is an early branching human enteric parasite that infects an estimated 50 million people per year and is a significant cause of morbidity and mortality. The genome of *E. histolytica* is 22.8 Mb in size and encodes about 9938 genes which comprise 49% of the genome. In *E. histolytica*, rDNA genes are located almost exclusively on extrachromosomal circular DNA molecules (Bhattacharya et al., 1988; Bhattacharya et al., 1992; Bhattacharya et al., 1989). The work presented in this thesis was carried out with the aim to identify and characterize various transcriptional components in the genome of *E. histolytica*.

The first part of this study focuses on the detailed analysis of RNA Pol I subunits in *E. histolytica* genome database. The results obtained are summarized below:

1. The two largest RNA Pol I subunits and their zinc-binding motifs were found to be highly conserved in *E. histolytica* when compared with other organism counterparts.
2. EhRPAC40 and 19 subunits contained alpha-motif.
3. Five common subunits RPB5, 6, 8, 10 and 12 were found to be conserved in *E. histolytica*.
4. RNA Pol I specific subunits EhRPA3, 4 and 12 were present in genome database.
5. Most of the RNA pol II and III subunits were identified in genome database.
6. Sequence analysis showed changes in key residues of α-amanitin motif and bridge helix domain in EhRPB1 which may contribute to the α-amanitin resistance in *E. histolytica*.

In the second part, Molecular cloning and characterization of many polypeptides belonging to various transcription complexes of *E. histolytica* was performed. The results are summarized below:

1. EhRPA1, 2, 12 were found to be transcriptionally active in *E. histolytica*.
2. Antibodies were raised against EhRPA1, 2 and 12 which recognized their respective native protein in *E. histolytica* nuclear extracts.
3. RNA Pol II subunit RPB9 and Pol III transcription factor BRF were found to be transcriptionally active in *E. histolytica*. Antibodies raised against these two recombinant proteins recognized their respective native protein in *E. histolytica* nuclear extracts.

4. EhTBP and EhTIFIA were cloned and expressed. Antibodies were raised against recombinant proteins which recognized native proteins in nuclear extracts. Both the proteins were found to be transcriptionally active.

5. Nucleolar marker protein fibrillarin was cloned and expressed. Antibodies were raised against this protein for nucleolar localization experiments.

The third part of this study involved understanding nucleolar organization in *E. histolytica*. The results obtained are:

1. Fibrillarin and three RNA Pol I subunits colocalized at the nuclear periphery confirming a peripheral nucleolar organization in *E. histolytica*.

2. RNA Pol II was present exclusively in nucleoplasm while Pol III was present predominantly in nucleoplasm with a minor component at the nuclear periphery

3. Transcription factor EhTBP was present in both nucleolus and nucleoplasm

4. Nucleolus remained intact during various stress conditions like, cycloheximide, heat, oxygen and serum starvation, although some redistribution of RNA Pol I and fibrillarin took place inside cytoplasm.

5. Treatment with kinase inhibitors rapamycin and staurosporine lead to major changes inside nucleus. Rapamycin treatment resulted in nucleolar foci formation and staurosporine treatment resulted in nuclear elongation.

6. Nucleolar changes associated with trophozoite to cyst transition in *E. invadens* showed a gradual breakdown of peripheral nucleolar ring into various foci.

The fourth part of this study involved Phosphorylation studies on RNA Pol I basal transcription factor EhTIFIA. The results obtained are:
1. EhTIFIA shows limited sequence conservation with other TIFIA s throughout the length of the protein

2. The phosphorylation sites identified in human TIFIA were found to be absent in EhTIFIA

3. EhTIFIA gets extensively phosphorylated in presence of *E. histolytica* nuclear extract

4. Multiple sites were found to be involved in EhTIFIA phosphorylation

5. Mass spectroscopy analysis of EhTIFIA phosphorylation showed three putative target sites of phosphorylation.