One of the significant advances in cell biology during the past decade is the convincing evidence that mitochondria are semi-autonomous structures capable of replicating themselves. These organelles have been demonstrated to contain a distinct species of DNA different from the nuclear counterpart and a characteristic protein synthesizing apparatus including different types of RNAs, ribosomes and a host of enzymes necessary for the transcription and translation of their genetic material. However, the information content of mitochondrial DNA is far from sufficient to code for all mitochondrial proteins. Except for a few insoluble proteins of the inner membrane, most are coded by nuclear genes, synthesized on cytoplasmic ribosomes and subsequently transferred to mitochondria. Evidence is recently accumulating to show a considerable coordination existing between the two protein synthesizing systems. A detailed study of the synthesis of certain mitochondrial enzyme complexes such as oligomycin-sensitive ATPase and cytochrome oxidase, using site specific inhibitors, has provided interesting information on the role of mitochondrial protein synthesis in the final assembly of these complexes. It has been shown that synthesis of the non-catalytic sub-units by mitochondria, which serve as anchor points for the catalytic sub-units synthesized on cyto-ribosomes, are obligatory to the final integration of the sub-units and subsequent expression of the enzyme activity. More information on such inter-relationship is necessary for a better understanding of the assembly of mitochondrial proteins. Site-specific
inhibitors like cycloheximide (CH) and chloramphenicol (CAP) serve as excellent tools for such studies.

Although considerable work on the foregoing lines has been done, the manner in which mitochondria are formed anew in the cell is not yet fully understood. Different hypotheses have been put forward to explain this mechanism: (a) mitochondrial formation from intra-cellular structures; (b) symbiotic origin from bacteria; (c) de novo synthesis; and (d) formation by growth and division of the pre-existing mitochondria. The last mode of formation is compatible with the recent consolidating evidence for the existence of DNA in mitochondria and is the most favoured hypothesis. The classical experiments of David Luck with cholineless mutant of *N. crassa* have provided convincing proof for mitochondrial formation by growth and division in these organisms. In yeast, extensive work has been done on the occurrence of undifferentiated promitochondria under anaerobic condition which evolve into fully developed mitochondria upon exposure to oxygen. Similar conclusions are reached from studies on *T. pyriformis* and on flight muscle of *Locusta migratoria*. Despite numerous studies carried out with unicellular organisms, little evidence is available regarding the genesis of mitochondria in mammalian cells.

Recently, considerable work has been done in this laboratory towards elucidation of the problem of mitochondrial biogenesis from a study of the heterogeneity of mitochondrial
population in rat liver. It has been observed that rat liver mitochondria can be resolved by well controlled procedures of differential centrifugation into three entities - the heavy, light and fluffy mitochondrial fractions - having distinct differences in their properties. A comparative study of the properties of these three mitochondrial fractions, under normal conditions as well as under conditions of stimulated protein synthesis by treatment of thyroidectomized rats with physiological doses of tri-iodothyronine (T3), have given interesting results. All the three fractions possess typical mitochondrial membrane-bound and soluble enzymes. Heavy mitochondria show good respiratory control and optimum ADP/O ratio. The fluffy fraction appears to be loosely coupled. However, the light mitochondria lack the capacity to carry out oxidative phosphorylation and are characterized by a high synthetic ability followed by rapid protein turnover. There is an early stimulation of succinoxidase activity in this fraction after T3 treatment as compared to heavy and fluffy mitochondria. Their proteins decrease by 40% on thyroidectomy and increase by 160% on T3 treatment whereas the changes in heavy and fluffy fractions are not significant. Results on protein turnover are also suggestive of a transfer of preformed protein units synthesized extra-mitochondrially to light mitochondria. These observations indicated that the light fraction may consist of premature mitochondria which develop into stable heavy mitochondrial structures, while the fluffy fraction may consist of degenerate forms. However, further characterization of these mitochondrial
fractions in respect of their nucleic acids profile, protein synthesis in vitro, lipid make-up and synthesis in vivo and electron microscopic observations were felt necessary in order to have a better understanding of the role of the light mitochondria in mitochondriogenesis. Studies on these aspects are presented in the first half of the thesis (Section II). The second half (Section III) deals with a study of the effect of CH on mitochondrial protein synthesis. This was of interest in view of the current information on the interdependence of the intra- and extra-mitochondrial protein synthesising systems. An introductory chapter (Section I) surveys the literature on these subjects and outlines the scope of the present work.

Each section includes details in respect of literature citations, methodologies and discussions independently of other sections. Even though this has entailed some repetition, this presentation has been prefaced to make the sections self-contained.

II.1. The significance of promitochondrial structures in rat liver for mitochondrial biogenesis

Rat liver mitochondrial population, separated into heavy, light and fluffy fractions by differential centrifugation, was further characterized with respect to their in vitro protein synthesizing abilities. The light mitochondrial fraction incorporates DL(1-14C) leucine in vitro into its proteins at a rate 4–5 times higher than the heavy and fluffy fractions; this
fraction also shows most significant changes in response to alterations in the thyroid status of the animals. The radioactivity in leucyl-tRNA of light mitochondria is also about 3–4 times higher than that of the other two fractions. Incorporation of label in all the mitochondrial fractions is inhibited equally to the extent of 52–54% by chloramphenicol, whereas CH, a known inhibitor of microsomal protein synthesis, does not have any significant effect. These results, together with the observations on an increased RNA content of light mitochondria, appear to be in agreement with its high protein synthetic activity in vitro.

DNA from heavy, light and fluffy mitochondrial fractions has been isolated, purified and characterized by CsCl density gradient centrifugation procedures. The DNA samples from all the three fractions show identical buoyant densities of 1.701 g/cm³; corresponding value for nuclear DNA is 1.703 g/cm³. These observations, besides emphasising the purity of these fractions, strongly suggest autonomy and physical continuity amongst them.

A direct proof for the existence of mitochondrial heterogeneity was obtained from electron microscopic observations on these three fractions. The heavy mitochondria appear to be well organized structures, with densely packed cristae and with intact double membranes. The light mitochondria are much smaller in size than their heavy counterparts, with less cristae space. Various stages of maturation are also seen in this fraction. These range from vacuolar structures surrounded by double membranes, in which
cristae are totally absent, to the rudimentary mitochondria with partially formed cristae, and to the relatively more mature forms with typical mitochondrial architecture. The fluffy fraction represents a mixture of heavy and degenerating mitochondria. Frequently, areas are seen where mitochondrial structures are completely disorganized with loss of outer membrane.

Presence of bud-like outgrowth is frequently observed in heavy mitochondria, such structures being absent in the light and fluffy fractions. This raises the question whether this is the mode of formation of the light mitochondria from the mature mitochondrial structures. This aspect has been separately dealt with in a supplement to this section.

II.2. Further characterization of rat liver mitochondrial fractions: Lipid composition and synthesis; characterization of proteins.

Since lipids as lipoproteins, form important major components of mitochondria, the heavy and light mitochondrial fractions were characterized with respect to their lipid composition and synthesis. Protein profile as seen by SDS-polyacrylamide gel electrophoresis was also examined. For comparison, parallel studies have also been carried out with microsomes.

The light mitochondrial fraction is rich in total lipids, phospholipids and cholesterol. The cardiolipin content is, however low. The rates of $^3$H-glycerol incorporation into phospholipids of
heavy mitochondria and microsomes are almost identical. Light mitochondria show about 4 to 6 times higher incorporation into its phospholipids.

Analysis of protein profiles by SDS-polyacrylamide gel electrophoresis shows that patterns obtained for light mitochondria are apparently similar to those of heavy mitochondria. However, the light fraction is relatively poor in high molecular weight proteins and rich in low molecular weight proteins. Microsomal protein profile is altogether different with respect to number of peaks and their molecular weights as well as percentage distribution of proteins.

Results of these studies taken together (Section II.1 and II.2) indicate that the three mitochondrial fractions are separate entities representing different phases of mitochondrial genesis and turnover, the young, the mature and the degrading form the same structure.

III. Study of protein synthesis in rat liver mitochondria: Use of cycloheximide.

Cycloheximide has been found to be a useful tool to distinguish between the processes of extra-mitochondrial and intra-mitochondrial protein synthesis. This antibiotic is a potent inhibitor of cytoplasmic protein synthesis in the yeast, Tetrahymena, Neurospora and also in several mammalian cells. In contrast, it
does not inhibit amino acid incorporation *in vitro* by isolated mitochondria. There are conflicting reports regarding the *in vivo* effect of this drug on mitochondrial protein synthesis. While there are observations to show that CH resistant protein synthesis occurs under *in vivo* conditions, some workers have also observed that CH *in vivo* inhibits total cellular protein synthesis, including that of mitochondria. Most of these observations are with unicellular organism and there are only a few reports pertaining to the use of this inhibitor in the study of protein synthesis in mammalian systems. An attempt has therefore been made to study the effect of CH on mitochondrial protein synthesis in rat liver both under *in vivo* and *in vitro* conditions.

Effects of short and long-term administration of CH on rat liver mitochondrial protein synthesis have been examined and are found to be differential. Long-term administration of CH resulted in inhibition of total cellular protein synthesis including that of mitochondria, while at short-term intervals, 8 - 10% of mitochondrial protein synthesis is CH resistant. The inhibitory effect is also reflected in terms of *in vitro* protein synthesizing ability of mitochondria; the inhibition becoming apparent at 40 min and showing progressive increase with time. The observed inhibition of mitochondrial protein synthesis by CH is not due to either inhibition of energy metabolism or alteration of amino acids pool. CH did not enter mitochondria or sonic preparation under *in vitro* conditions. On the other hand, after
administration of $^3$H-CH significant quantities of $^3$H-CH are found to be associated with mitochondria and mito-ribosomes. These results indicated that CH reached the site of action in mitochondria, under in vivo conditions but was unable to do so in vitro.

These studies reveal that inhibition by CH of mitochondrial protein synthesis may be due to the effect of CH on mitochondrial protein synthesizing system per se or may arise out of interdependence of mitochondrial and microsomal protein synthesis.