3. OBJECTIVES

The main focus of this work is to investigate the role of PtdIns in Melanogenesis, and effect of para-Phenylenediamine on melanin producing cell. Tyrosinase, a key enzyme in the melanogenesis pathway, undergoes maturation, intracellular transport in the classical secretory pathway which involves ER, Golgi apparatus and endocytic machinery. PtdIns are long been implicated in the intracellular trafficking particularly the endocytic system.

para-Phenylenediamine (p-PD) was long been known to be an essential component of permanent hair dyes. p-PD is used in textile, leather and especially as a key ingrediant in hair dye industries. Studies showed that use of p-PD can increase the occurrence of liver, kidney, and urinary bladder and thyroid gland tumors in rats (Sontag, 1981). Partially oxidized states of p-PD can cause allergy in sensitive individuals. Nothing much is known about its effect on melanin producing cells.

There are two major objectives in this study:

Objective A: **"Role of PtdIns 4P 5 kinase type I α (PIPK) in Tyrosinase biogenesis in A375 cells".**

For the ***Objective A*** we took the following approaches:

1. Generation of A375 cell lines stably expressing wild type and kinase dead FLAG-tagged PIPKs.
2. Determination of sub-cellular localization of Tyrosinase and FLAG-PIPK by immunofluorescence in the A375 cell lines using anti Tyrosinase and anti FLAG antibodies.
3. Confirmation of ER localization of Tyrosinase by double immunofluorescence using antibodies of Tyrosinase and Protein Disulfide Isomerase (PDI; ER marker)
4. Co-immunoprecipitation to prove the association of Tyrosinase and PIPK in the cellular fractions.
5. Quantitative analysis of Tyrosinase transfer to the post ER/Golgi fraction.
6. Transient transfection of different A375 cell lines by different PH/FYVE domains tagged with GFP to to determine the role of endogenous PH domain containing proteins in Tyrosinase maturation.

Objective B: **"Effect of para-Phenylenediamine (p-PD) on Human Melanoma cells (A375)"**

For the ***Objective B*** we have performed the following experiments:

1. Measurement of the Melanin content and the Tyrosinase activity level in p-PD treated A375 cells.
2. Determination of cytotoxicity of p-PD on human melanoma (A375) by MTT based assay.
3. Nuclear staining by DAPI and western blot analysis using apoptotic marker proteins to find out the mechanism of p-PD mediated cell death
4. DCFH-DA staining and Rhodamine 123 staining to see that status of mitochondrial ROS and its potential during cell death.