Pax3 is a transcription factor crucial for normal development. It contains two DNA-binding domains, a paired domain (PD) at the N-terminus and a paired-type homeodomain (HD) at the C-terminus. It has been shown that point mutations, which mostly located in the PD or HD, cause Waardenburg syndrome, and chromosomal translocations that create Pax3-FKHR fusion proteins cause alveolar rhabdomyosarcoma. We found that Pax3 used its PD and HD to interact with proteins known to associate with or to regulate the function of chromatin, and it associated with heterochromatin during interphase and with mitotic chromosomes during mitosis. Furthermore, a mutant Pax3 from Waardenburg syndrome patients was de-localized from heterochromatin and the mitotic chromosome. Recent studies show that proteins localized to heterochromatin and the mitotic chromosome participate in interchromosomal interactions. Therefore, I hypothesize that Pax3 associates with heterochromatin and the mitotic chromosome, leading to interchromosomal interactions that are de-regulated in Waardenburg syndrome and rhabdomyosarcoma. The following three aims are proposed to test this hypothesis:

Aim 1: to determine how Pax3 associates with heterochromatin and the mitotic chromosome using domain-mapping, protein-protein interaction assays, and chromosome spreading assays.

Aim 2: to determine if Pax3 mutants from patients with Waardenburg syndrome and rhabdomyosarcoma are associated with heterochromatin and the mitotic chromosome using confocal microscopy, protein-protein interaction assays, and chromosome spreading assays.

Aim 3: to determine if Pax3 functions in interchromosomal interactions through chromosome conformation capture (3C) and chromatin immunoprecipitation (ChIP) assays.

This proposed study will demonstrate the mechanism by which Pax3 interacts with heterochromatin and the mitotic chromosome and the significance of Pax3 in interchromosomal interactions, which might explain how mutations in Pax3 cause Waardenburg syndrome and alveolar rhabdomyosarcoma. Results from this study will help us further understand the normal function of Pax3 and lead to possible therapeutic interventions in the future.
**Keywords:** Pax3, Waardenburg syndrome, alveolar rhabdomyosarcoma, KAP1, HP1, subnuclear localization, interchromosomal interaction, chromosome spreading, 3C, ChIP