CHAPTER 20

GENETICS

Doctoral Theses

01. CHAUDHURI(Zia)
Genetic Analysis of Strabismus.
Supervisor : Prof. S. Aneja and Prof. B. K. Thelma
Th 23458

Abstract
(Not Verified)

Primary concomitant strabismus (PCS), implying misalignment of the eyes, is an important childhood morbidity comprising esotropia (ET) and exotropia (XT). It exists as sporadic or familial forms, latter suggestive of a genetic basis. Only three chromosomal loci have been linked to PCS but they do not explain all tested families. Identification of additional genetic determinants for this condition using next generation sequencing approach was the aim of this doctoral study. Recruitment of informative PCS families was the first objective. 39 families thusrecruited comprised of 18 ET, 18 XT and 03 with both, with more vertical transmission seen inXT (Chaudhuri et al, 2017), making it the choice for further studies.Identification of known/novel variant(s) causal/associated with XT was the second objective andtwo informative families were selected for whole exome sequencing (WES). In family #4, with presumptive autosomal dominant inheritance pattern, all five affected and three unaffected members were sequenced and three rare heterozygous missense variants, one each in *EPHA2*,*NUP160* and *STIP1* segregating with the phenotype were identified. Based on availableliterature and interaction network analysis done in this study, *EPHA2*(1p36.13*)* with a raremissense variant (1:16451737;c.2904G>C:p.Gln968His; MAF=0.0007[ExAC SAS]) emerged as astrong candidate for XT in this first report. Of note, Sanger sequencing of all 17 exons of *EPHA2*in an independent PCS cohort identified two additional rare variants. In family #14, with anunclear inheritance pattern, WES data of three affected and three unaffected members did notidentify segregating variant(s). On revisiting clinical details and considering only one arm of thefamily, with likely autosomal recessive inheritance with two unaffected and two affected siblingsshowing foveal hypoplasia and nystagmus, a homozygous stopgain mutation, (c.264 C>G:pY88*;MAF=0.00006[ExAC SAS]) in *SLC38A8*(16q23.3) segregated with the phenotype. Thesepromising findings encourage further discovery genomics, trans-ethnic replication studies andfunctional validation.

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02. Kritika RAJ

Studies on the Role of Insulin Signaling and its Association With myc in Alleviating the Human Poly (Q)-Mediated Neurodegeneration in Drosophila.

Supervisor: Prof. Surajit Sarkar

Th 23460

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1. Introduction. 2. Materials and methods 3. Tissue specific upregulation of the Drosophila insulin receptor (Inr) mitigates poly (Q) mediated neurotoxicity by restoration of the cellular transcriptional machinery 4. Concureent upregulation of inr and dmyc additively alleviates poly (Q) mediated neurotoxicity and the conserved transactivation domain of c-myc/dmyc is essential for driving the rescue event. 5. Summary. References. Annexures.

03. LAXMI

Identification of Rare Putative Causal gene Variants in Intellectual Disability and Parkinson's Disease Families.

Supervisor: Prof. Surajit Sarkar

Th 23756

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1. Review of literature and introduction 2. Materials and methods 3.A novel homozygous mutation arg459 pro in synj1 gene 4.A de novo detetion at 13q14.2-21.1 in two siblings with id 5.An unsual family with a secket syndrome phnotype.Summary and perspectives. Appendices..References.

04. MEHRA (Upasana)

Fuctional Characterization of a gtpase yor205c (mtg3), a Mitochondrial Ribosome Assoiated Factor in Saccharomyces Cerevisiae.

Supervisor: Prof. KaustuvDatta

Th 23455

Abstract
(Not Verified)

Mitochondria are made up of proteins encoded by the nuclear genome as well as its own genome, thusrequiring both cytosolic as well as its own translation apparatus for its biogenesis. The proper biogenesis of mitochodria is critical as 1 in 5,000 humans suffers from a mitochondrial disease and a number ofthese diseases are due to defects in the mitochondrial translation apparatus. Importance ofmitochondrial translation apparatus can be estimated with the fact that approximately 25% of themitochondrial proteome is involved in the establishment and the maintenance of the mitochondrialprotein synthesis apparatus and mitochondrial DNA. Mitochondria utilize dedicated ribosome moleculesthat are encoded by a set of nuclear genes distinct from those coding for its cytosolic counterpart.Ribosome biogenesis is a multi-step process aided by assembly factors including GTPases which arethought to utilize energy released upon GTP hydrolysis to promote its biogenesis activity. Mtg3p, is anuclear encoded mitochondrial protein that is conserved from yeast to humans and is a member ofYawG/YlqF family of circularly permuted GTPases. Deletion of *MTG3* leads to defects in utilization ofglycerol as the sole carbon source and accumulate 15S rRNA precursors. I have shown that Mtg3p cofractionates with both small and large ribosomal subunit in a salt dependent manner on a sucrosegradient and has the ability to form a complex with Mtg2p, a 54S assembly factor suggesting Mtg3pfunctions beyond a small mitoribosomal subunit biogenesis factor. Consistent with our association datacells expressing *ts*alleles of *MTG3* are defective in large subunit biogenesis. Taken together, ourstudies indicate that Mtg3p functions at a late step in the

biogenesis perhaps just prior to associationwith mRNA to form 74S monosome. In other words, *MTG3* plays a role in coordinating both small andlarge subunit biogenesis.

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1. Introduction. 2. Mtg3 is essential for mitochondrial function and associates with the mitochondrial ribosomes 3. Mtg3p play a role in coordinating mitochondrial ribosomal subunit biogenesis 4. Mtg3p associates with mitochondrial ribosomes via its c-terminus and requires its putative gtpase activity for in vivo function.5. Conclusions. References.

05. NEMNEINENG HAOKIP
Understanding the Importance of ParvulinLike Peptidyl Cis-trans Isomerase, pinA in DictyosteliumDiscoideum.
Supervisor: Prof. P. K. Burma
Th 23456

Contents

1. General Introduction objective of the work 2.Materials and methods 3. D. discoideumpina and pin4 genes: Cloning and complementation in s. cerevisiae 4. Wild type studies of pinA 5. pinA function: Oveexpression, knock – out and their phenotypic analyses 6. Summary and future perspective. Appendix. References. Publication.

06. PANDEY (Dharmendra Kumar)
Characterization of the Potential Role of a Putative Mitochondrial Gtpase ydr336w, in Regulating Mitochondrial Function in Saccharomyces Cerevisiae.
Supervisor: Dr. KaustuvDatta
Th 23459

Abstract
(Not Verified)

Mitochondria are central to metabolism, intracellular and organellar communication besides being thepowerhouse of the cell where majority of ATP is synthesized. *YDR336w* belong to YihA family ofproteins, conserved in bacteria, lower eukaryotes such as *S. cerevisiae*, *C. albicans*, and vertebratesincluding humans. Interestingly, it is absent from all other forms of eukaryotic life sequenced so far. Thisobservation suggests that a branch of mitochondrial function controlled by *YDR336w* is conserved inlower eukaryotes and vertebrates but is dispensable for all other eukaryotic life forms. *Bacillus subtilis*YsxC (termed YihA in *E. coli*) is essential for the growth and cells depleted for YsxC accumulateimmature ribosomal subunit intermediates.Ydr336wp is predicted to be localized to themitochondria.Ydr336wp is largely conserved with its bacterial family member with an additional 132amino acids at its N-terminus predicted to contain a mitochondrial targeting sequence. We have shownthat cells deleted for *ydr336w* grew slowly in comparison to wild type yeast cells on glycerol as the solecarbon source at lower temperature. I have also shown that Ydr336wp is peripherally localized to theinner mitochondrial membrane facing the matrix side. Ydr336wp tightly associates with the mitochondrialribosome on the large subunit face. In addition, mutations that abolish GTP and GDP binding were alsonot able to support cellular respiration; while mutations that are predicted to lock the protein in either GDP or GTP state were. This indicates that *in vivo* Ydr336wp functions to communicate the availability ofNTP or NDP to regulate mitochondrial function. Heterologous expression of yeast MTS tagged humanorthologue of Ydr336wp in *Δydr336*w cells as a sole allele for *YDR336w* was able to support growth ofyeast cells on glycerol as sole carbon source, indicating a conservation of *YDR336w* function in both

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1.Introduction 2. Ydr336w is important for optimal cellular respiration 3. Ydr33wp Associates with mitochondrial ribosomes 4. Gtp/gdp binding is critical for ydr336w function in vivo 5. Conclusions

07. PANDEY(Namita)
Studies on risk Analysis, Molecular Signaling and Therapy for non-small Cell Lung Cancer.
Supervisor: Prof. Anant Mohan and Dr. Tapasya Srivastava
Th 23462

Abstract
(Verified)

In an effort to encompass various aspects of basic and pre-clinical lung cancer research, the study investigates risk analysis, molecular signaling network and therapy for non-small cell lungcancer. Two SNPs rs16969968 and rs3743074 of 15q25 region analysed as risk marker for lungcancer and nicotine dependence, and previously unanalysed in Indian populations, showedsignificant association of rs16969968 with both conditions.15q25 locus contains genes encoding subunits of nicotine acetylcholine receptor (nAChR), animportant contributor to lung cancer progression. The cells in the hypoxic tumormicroenvironment are known to be more aggressive and resistant to therapy. We have studiedthe crosstalk betweennAChRs mediated signaling pathway and hypoxia regulated pathways and observed an increase in acetylcholine and nAChR subunit α7 level in hypoxic lung cancer cells,in turn regulating the expression of hypoxia inducible factors (HIFs) to help cancer cells adapt tohypoxic condition.We have also explored the potential of a naturally derived plant molecule as therapy in theheterogeneous tumor microenvironment. A novel ROS mediated molecular mechanism of allicin(a key organosulfur compound present in garlic) induced cell death in lung cancer wasestablished and its efficacy in heterogeneous tumor microenvironment was determined.Furthermore, the antitumor potential of three transition metal oxide nanoparticles namely copperoxide (CuO), nickel oxide (NiO) and ferric oxide (Fe O) against lung cancer was investigated innormoxia and hypoxia. This is the first study to analyze the mechanism of anti-cancerousproperties of these metallic oxide nanoparticles in the hypoxic tumor microenvironmentOverall, the thesis is an attempt to evaluate various aspects of lung cancer biology relevant to theIndian population and offer new insight into molecules of potential therapeutic value.

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1.Deterninig association of snps rs16969968 and rs3743074 at 15q25 locus with lung cancer and smoking status in Indian Population 2. Investigating the cross talk between nicotine acetyl choline receptors (nachrs) and hypoxia inducible factors (hifs) mediated signaling pathway in hypoxic lung tumor microenvironment 3. Determining the cellular and molecular mechanism of cytotoxicity of natural compound allicin in non-small cell lung cancer (nscls) and its effectiveness in combination with standard-of-care drug, cisplatin 4.Assessing the antitumour potential of transition metal oxide nanoparticels against lung cancer in hypoxic tumor environment.Summary of the thesis.References.Appendices.Publications.

08. SHARMA(PreetiApurve)
Characterization of Tapetum Specific Promoter ta 29 from Nicotianatabacum.
Supervisor: Prof. Pradeep Kumar Burma
Th 23457

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1.Introduction 2. Re-evaluation of ta29 promoter activity in transgenic tobacco plants 3. Studies on regulation of ta 29 promoter. 4. Materials and methods. Annexure. Publication

09. VERMA (Neetu)
Analysis of Tapetum Specific Promoter a9 from Arabidopsis Thaliana.
Supervisor: Prof. Pradeep Kumar Burma
Th 23461

Abstract
(Verified)

Tapetum specific promoters like *A9* from *A. thaliana* and *TA29* from *Nicotianatabacum*have beenwidely used for hybrid seed production. Inspite of their usage in plant biotechnology, not much workhas been carried out to understand the regulation of such promoters. Regulation can be achieved bypositive regulators activating the promoter in the target tissue or negative regulators keeping thepromoter repressed in all other tissues except target tissue or by a combination of both positive andnegative regulators. The present study aimed to characterize the tapetum specific promoter *A9.* Thisstudy was initiated with an *in silico* analysis of promoters of the A9 gene and those that are coexpressedwith it, to predict the regulators and their *cis*-elements. Out of the predicted regulators,two transcription factors (TFs) AtMYB80 and AtMYB1 were proposed to be positive regulators andone TF, AtMYB4 was proposed as a negative regulator. The hypothesis was evaluated usingdifferent strategies in *A. thaliana* and in anhetrologous system *N. tabacum*. The role of positiveregulators, AtMYB80 and AtMYB1 was studied by generating overexpression transgenic lines or bytransient expression strategies. Our results showed that AtMYB80 and AtMYB1activated *A9* promoter at the ectopic location. This observation was substantiated by mutating theputative *cis*-elements for these regulators and observing the loss of *A9* promoter activity. This studyalso identified two *cis*-elements through which AtMYB80 and AtMYB1 bring about their effect. Therole of the negative regulator, AtMYB4 was studied by downregulating the expression of thistranscription factor in transient assay system and by mutating the putative *cis*-elements for AtMYB4.Our results in *A. thaliana* indicated that AtMYB4 may not be involved in the regulationof *A9* promoter. However in case of tobacco, mutation of *cis*-elements led to activation of promoterin root tissue, suggesting that a transcription factor in tobacco similar to AtMYB4 is probablycontrolling *A9* promoter in the root tissue.

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1.Introduction 2. Results 3. Discussion 4. Matterials and Methods 5. Bibliography. Annexure.

10. YOGINDRAN (Sneha)
Artificial microRNA – mediated silencing of ecdysone receptor gene of helicoverpaarmigera for insect resistance in tomato.
Supervisor: Prof. M. V. Rajam
Th 23463

Abstract
(Not Verified)

Tomato is one of the important vegetable crops grown and consumed worldwide. It is a rich source of an antioxidant, lycopene,implicated to control various diseases. However, tomato is affected by several environmental factors, pest infestation and diseases, whichseverely influence its growth and

development leading to significant yield loss. *Helicoverpaarmigera*is one of the major pests of tomato and can completely destroy the plant. The present study deals with the use of an artificial miRNA (amiRNA) strategy for the control ofthis notorious insect pest by targeting its *Ecdysone receptor* (*EcR*) gene. Ecdysone or 20-hydroxyecdysone is an insect steroid hormone,which binds to its receptor (*EcR*) and plays important roles during development and metamorphosis. The precursor miRNA mse-let-7aof *Manducasexta*carrying the amiRNA sequence was cloned into bacterial feeding vector L4440 and expressed in *E. coli* under T7promoter for *in vitro* insect bioassays. Feeding bioassay with amiRNA-let7a-*HaEcR* construct showed reduced target gene transcripts andimpaired insect growth and oogenesis as compared to controls. Taking cues from the *in vitro* feeding assays, the amiRNA-*HaEcR*wasfurther cloned in the precursor backbone of ath-miR-319a of *Arabidopsis thaliana* for *Agrobacterium*-mediated tomato transformation.Insect feeding assays with detached leaves of T_0 and T_1tomato transgenic lines showed enhanced resistance to *H. armigera*. It affected thelarval growth and resulted into significant mortality. The target gene expression level was also considerably reduced in transgenic leavesfed larvae as compared to the control. These results suggest that the insect precursor miRNA backbones can be used for successful geneknock-down studies in insects and plant expressing amiRNAs against vital genes of the target insect pest can be effectively used as apotential and efficient tool along with the existing approaches to control the pest population.

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