CHAPTER 18

GENETICS

Doctoral Theses

01. ARORA (Heena)

Isolation and Characterization of White Rust Resistance Genes in *Brassica juncea.*

Supervisor : Prof. Akshay K. Pradhan

Th 25214

Abstract

(Not Verified)

White rust caused by oomycete pathogen Albugo candida is a significant disease of crucifer crops including Brassica juncea, a major oilseed crop of Indian subcontinent. This study was conducted with primary aim of controlling the disease in Indian mustard lines through genetics-based host and nonhost resistance strategies. Earlier, host resistance-conferring locus AcB1-A5.1 was mapped in an east European gene pool line of B. juncea – Donskaja-IV using A. candida isolate AcB1. This line was tested along with other lines of B. juncea, B. rapa and B. nigra for resistance to isolates of A. candida collected from different mustard growing regions of India. Donskaja-IV was completely resistant to all the tested isolates. Sequencing of BAC spanning the locus AcB1-A5.1 showed the presence of a single CC-NLR type of R gene. Genomic sequence of putative R gene with its native promoter and terminator was used for genetic transformation of susceptible Indian gene pool line Varuna and was found to confer complete resistance to all the isolates. This is the first white rust resistance-conferring gene described from Brassica species and has been named BjuWRR1. Allelic variants of gene in B. juncea germplasm and orthologues in Brassicaceae genomes were studied to understand evolutionary dynamics of BjuWRR1 gene. Additionally, functional characterization of a nonhost TIR-NLR type of white rust resistance-conferring R gene WRR4A from Arabidopsis thaliana in a susceptible Indian gene pool line of B. juncea was carried out. We have demonstrated that WRR4A when transferred to Indian B. juncea line Varuna provides high level of resistance against Indian isolate of A. candida AcB1. The levels of resistance varied in transgenic lines with line having multiple copies of WRR4A consistently showing high levels of resistance. The WRR4A gene could be an additional source for developing lines with durable resistance by stacking it with BjuWRR1 gene.

Contents

1. Introduction and review of literature 2. Map based cloning functional characterization and evolution of host white rust resistance gene *BjuWRR1* from B. juncea 3. Functional characterization of a non host white rust resistance gene *WRRS* from *A. thaliana* in *B. juncea* 4. Summary and future perspectives. Bibliography. Annexures.

02. KAUR (Jaswinder)

Regulation of Mitochondrial Function by a Yeast Clade Specific Putative Helicase *IRC3* in *Saccharomyces cerevisiae.*

Supervisor : Dr. Kaustuv Datta

Th 25213

Abstract
(Not Verified)

Mitochondrial gene expression requires dedicated translation machinery distinct from its cytosolic counterpart. Ribosomal proteins as well as their regulators are either universally conserved in eukaryotes or those that are species specific. Species specific proteins in the mitochondrial ribosome/regulators are predicted to increase the ability of the mitochondrial ribosome to appropriately respond to niche specific carbon and environmental cues. DEAD/H box helicases are universal regulator of process requiring RNA molecule including splicing, translation and ribosome biogenesis. IRC3 is DEAD/H box protein that is essential for cellular respiration. I have shown that upon deletion of IRC3, cells rapidly lose the ability to utilize glycerol as the sole carbon source followed by loss in intact mtDNA. I have also shown that Irc3p co-fractionates partially with both the small and large subunit of the mitochondrial ribosome on a sucrose gradient. Consistent with a role in mitochondrial translation, cells harbouring temperature sensitive alleles of irc3ts have reduced rates of translation, without having any consequence on the levels of mRNA transcript or assembled mitochondrial ribosomal subunit, when shifted to the non-permissive temperature when grown in glucose. Interestingly the reduction in mitochondrial translation is seen in Δirc3 but not irc3ts cells which are grown in galactose. Our results indicate that Irc3p regulates mitochondrial translation in cells but its mechanism of action is different under conditions that maintain mitochondrial function at a basal level versus under conditions that have higher mitochondrial function. Interestingly, it has been shown in vitro that Irc3p has an ATPase activity that is stimulated by DNA, and Δirc3 cells accumulated double stranded breaks in the mtDNA. Thus an alternate possibility is that function of Irc3p communicates mitochondrial translation status with the ability correctly repair/replicate mtDNA under conditions that require elevated mitochondrial function.

Contents

1. Introduction 2. *IRC3* is essential for mitochondrial function 3. *IRC3* is essential for mitochondrial translation during fermentative growth 4.*Irc3p* is associated with mitochondrial ribosome and suppressors of Δirc3v have altered translation rate 5. Conclusion and future directions. Appendix.

03. MAHAJAN (Divyank)
Targeting the Tumor Microenvironment Quest for Novel Targets for Cancer Therapy.
Supervisor : Dr. Tapasya Srivastava
Th 25531

Abstract
(Verified)

Hypoxic tumors contribute to drug resistance, cancer recurrence, and poor prognosis. Overcoming these requires understanding of the cellular and molecular behavior of hypoxic cells to identifying avenues of novel therapy and biomarkers. We have taken an unbiased approach to profile changes in the proteome and phosphoproteome of the lung cancer cells (A549) in response to 1% and 3% hypoxia, to understand mechanistic underpinnings of the adaptation programs undertaken by such cancer cells. Our quest was to model kinome reprogramming induced by hypoxia that alters the sites of drug binding and give rise to a refractory tumor. We explored the biological pathways, molecules and networks that arose from this model. Comparative proteome and phosphoproteome profiling of A549 cells

growing in hypoxia and normoxia revealed involvement of upregulated processes involved in intra-cellular transport and downregulation of repair processes, and mitochondria activities. This study builds a strong base for understanding the biology of the heterogenous lung cancer, and developing therapeutic modalities that address tumor hypoxia. We also assessed effect of Withaferin A in lung cancer cells and in Temozolomide resistant glioblastoma cells. We report that Withaferin A is equally effective in inhibiting cancer cell proliferation in both normoxia and hypoxia. We show that Withaferin overcomes hypoxia-induced advantages of the cancer cells thus inhibiting their growth and metastasis. Overall, the work has standardized and established proteomics work in the lab and paved the way for further functional and mechanistic studies based on the identified network and molecules. Additionally, in continuation with the lab interest in exploring anti-cancer potential of plant based natural products, the work puts forth a novel mechanism by suppression of hypoxia signaling pathway, to explain the effectiveness observed by us and other researchers working on Withaferin A.

Contents

1. Review of the problem 2. Material and methods 3. Exploration of the proteome and phosphoproteome of a non small cell lung cancer cell line A549 identify novel targets against hypoxic tumor microenvironment 4. Studies on anti cancer potential of withaferin A in the hypoxic microenvironment. Summary of thesis. References and appendices.

04. PANDEY (Anuj Kumar)

Characterization of ADP Ribosylation Factor Like Gtpase 15 (*ARL15*) In Rheumatoid Arthritis Synovial Fibroblast Cell Line Using an Integrated Omics Approach.

Supervisor : Prof. Akshay K. Pradhan

<u>Th 25216</u>

Abstract
(Not Verified)

Rheumatoid arthritis (RA) is a common complex autoimmune inflammatory disease characterized by chronic inflammation of the joints and caused by gene-environment interactions. Over 100 susceptibility genes/loci identified from transethnic population studies fail to explain missing heritability and disease biology, encouraging continued investigations. ADP ribosylation factor-like GTPase 15 (ARL15) is a novel non-HLA susceptibility RA gene identified previously in the lab but largely under-investigated at present. Therefore, two questions namely i) what is the role of ARL15 in target cells and ii) how does it contribute to RA biology, were addressed in this study. To start with genotype-phenotype correlation was established between the disease-associated ARL15 intronic SNP rs255758 (A>C) homozygous variant genotype (CC) and increased ARL15 protein levels in RA patients, with notable clinical relevance. Subsequent characterization of ARL15 using a gene knockdown strategy in rheumatoid arthritis synovial fibroblast cell line combined with hypothesis-free strategies revealed i) IL1b and TNF independent regulation of ARL15 (based on gene-expression microarray); ii) 56 differentially expressed genes mostly from the inflammatory pathways from transcriptomic sequencing and iii) 268 proteins of notable functional relevance from proteome analysis. Most importantly, a total of 25 putative interacting partners for ARL15 were identified by analyses of these datasets together with the data from ARL15 pulldown of cellular proteome using pathway analysis. Of note, ARL15 seems to have a role in multiple cellular processes such as inflammatory response, immunological disease, cancer, infectious diseases, cellular assembly and

organisation, cellular function and maintenance, amino acid metabolism, protein synthesis and molecular transport, which are in line with the function of other known ARL family of proteins. These to the best of our knowledge are first-ever findings on the role of ARL15. However, its direct or indirect contribution to RA etiology requires additional experimental investigations such as overexpression studies.

Contents

1. Review of literature and introduction 2. Material and methods 3. An intronic SNP (rs255758 A>C) alters the protein level of ARL 15, a novel rheumatoid arthritis gene among north Indians 4. Characterization of ARL15 in a RASF (MH7A) cell line using a knockdown strategy and multiple assays 5. Identification of ARL15 interacting partners by pulldown approach 6. Integrated analysis & summary and perspectives. References. Publication.

05. SAXENA (Shipra)
Development of Transgenics in Brinjal (*Solanum melongena L.*) for Resistance to Fruit and Shoot Borer by Using Rnai Strategy.
Supervisors : Prof. P. K. Burma and Prof. M.V. Rajan
Th 25217

Abstract
(Not Verified)

Brinjal, an agronomically important crop in India, is severely infested by insect pest, L. orbonalis. In spite of various control methods, including Bt approach, the pest is majorly controlled by routine application of insecticides. Recently, RNAi, has emerged as an environment friendly crop protection strategy. In the current study, CHSA, has been selected as a target gene for employing RNAi in the control of L. orbonalis. As a prelude to this investigation, initially RNAi has been employed for functional validation of CHSA in L. orbonalis, and subsequently tested the Host Inducing Gene Silencing (HIGS), producing RNAi transgenic brinjal plants expressing LoCHSA specific dsRNA for control of L. orbanalis. During the course of study, partial fragments of LoCHSA & LoCHSB isoforms detected in most insect species belonging to catalytic domain were isolated. Down-regulation of LoCHSA in L. orbonalis larvae by feeding artificial diet supplemented with E. coli expressing dsRNA led to prolonged larval period, reduced fecundity and reduction in transcript levels of LoCHSA as well as LoCHSB. Further, brinjal transgenics expressing LoCHSA-specific dsRNAs were generated by Agrobacterium-mediated transformation, and feeding of fruits from such RNAi lines led to a significant reduction in transcript levels of LoCHSA and LoCHSB. Also, SID-1, was found to be up-regulated in both the feeding assays, viz., after feeding upon dsRNA. Insect culture of L. orbonalis was established on natural and artificial dietary sources under controlled growth conditions. EF along with COI were found to be stable reference genes across different developmental stages under variable experimental conditions like dietary source and dsRNA exposure to L. orbonalis. This study thus provided an important insight for the use of RNAi for not only in functional genomics studies in L. orbonalis but also for control of the insect pest by targeting LoCHSA through HIGS.

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion 6. Summary and conclusions. References. Annexures

06. SHARMA (Aditya)

Drug Target Prioritization and Lead Molecule Development for Rheumatoid Arthritis.

Supervisor : Prof. B.K. Thelma

Th 25211

Abstract
(Not Verified)

Rheumatoid Arthritis (RA) is a common multifactorial disorder leading to severe joint deformity, disability and poor quality of life. Characterised by unknown aetiology and clinical and genetic heterogeneity, therapeutic management of RA is mostly limited to symptomatic treatment. However, non-responders, adverse drug reactions, high costs etc. pose continuing challenges and highlight the dire need for novel, orally ingestible drugs. To achieve this the current study aims to i) identify and prioritize druggable targets for RA, ii) identify and design novel lead molecules against prioritised target(s) using multiple computer aided drug design (CADD) approaches and iii) computational and experimental validation of their inhibitory potentials. A pool of >100 potential RA drug targets was compiled including genes from in-house genome wide association studies (GWASs) on north Indian cohorts and via exhaustive literature survey. These were filtered based on their amenability for CADD, leading to the identification of Bruton's tyrosine kinase (BTK) as the top priority target. Using the ligand-based CADD approach of pharmacophore modelling in conjunction with structure-based virtual screening identified chestanin and 3,3'-Digalloylprocyanidin-B2 as potential BTK inhibitors (Sharma and Thelma, 2019). Simultaneously fragment-based de novo ligand design was used to design novel compounds centred on a cinnoline scaffold, of which 18 were synthesized and tested for BTK inhibitory potential using a B cell line. Five of these with promising potency were assessed via a kinase assay and noted to exhibit excellent IC50 (<50nM) against BTK. Subject to further optimization and testing, these hold promise as potential lead molecules. In addition, ARL15, encoding for a small GTP-binding protein, identified as a novel RA susceptibility gene in the lab GWAS and with a likely therapeutic role was also explored. A high-quality 3D structure model has been predicted using ab initio method along with prediction of its GTP-binding site for future CADD studies.

Contents

07. SONI (Kamlesh Kumar)

Studies on Tapetum Specific Promoters from Cotton and Tobacco.

Supervisor : Prof. Pradeep Kumar Burma

Th 24212

Abstract
(Not Verified)

The work embodied in this thesis deals with two different promoters expressing in tapetum, one from cotton-AEG1 and other from tobacco-TA29. In a previous work, the1.5 Kb Upstream Regulatory Module (URM: promoter plus 5´UTR) of AEG1 showed activity in the tapetum layer as well as in roots of cotton transgenics. The present work re-engineered the AEG1 URM to make it tapetum specific. This was achieved by removing several root specific motifs identified in the URM. The importance of this work is in demonstrating that URM with activity in different tissues can be modified to make it tissue specific. That, the modified URM, AEG1(ΔBmut) is tapetum specific has been demonstrated by developing transgenics in tobacco using ß-glucuronidase as reporter gene. It has been further demonstrated that this URM can be used to develop male sterile lines in tobacco. AEG1(ΔBmut) thus opens up the possibility of developing barnase/barstar genes-based hybrid seed technology in cotton. Tapetum specific TA29 URM identified in 1990 has been extensively used for plant biotechnology purposes mainly to develop hybrid seeds. This URM however has not been characterized in terms of cis-elements and transcription factors (TFs) that make it tapetum specific. Taking cues from earlier work in the laboratory, the current work has demonstrated that cis-elements binding to TFs GT1-a, NAG1, MYBAS1 and LZHD regulate the TA29 URM. Of the four TFs, the first three acts as positive regulators while the fourth, LZHD is a negative regulator. While the above work was based on identifying the elements by in silico analysis and literature survey, work has also been initiated to identify novel elements through Linker Scanning Mutagenesis. Thus, this part of the work throws light on how tapetum specific promoters could be regulated.

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08. SUMEET KUMAR
Discovery of WASL and SCYL2 as Putative Disease Casual Genes and Mutation Spectrum in Known Genes in Parkinson Disease.
Supervisor : Prof. B.K. Thelma
Th 25215

Abstract
(Not Verified)

Parkinsonism is a common neurodegenerative disorder with limited treatment options and almost no cure. >20 genes identified using Mendelian forms of this group of disorders have uncovered significant genetic heterogeneity including in sporadic cases and a varying burden across populations. Total heritability however remains unexplained, encouraging major efforts in disease causal gene discovery. This study aimed at i) determining the contribution of known PD genes to an early onset PD cohort of Indian ancestry; and ii) identification of novel putative disease causal gene variant(s) using informative Parkinsonism families. Towards the first objective, genetic analysis of 250 well-characterized PD cohort using whole exome sequencing (WES) strategy, uncovered 64 patients with previously reported and 16 with novel variants together accounting for 32% of the study cohort; and revealed a significantly higher genetic burden ($p<0.0001$) in the PD cohort in comparison to a non-PD cohort ($n=616$) (Kumar et al., ms under revision). Discovery genomics efforts in four Parkinsonian families identified i) compound heterozygous variants in WASL in a family with a likely autosomal recessive mode of inheritance. WASL is a regulator of actin polymerization, and in vitro assays demonstrated that one variant leads to exon skipping and another alters the structure and function of neuronal networks; ii) In two other families, putative disease causal variants in SCYL2, a coated vesicle-associated kinase, were observed. Functional characterization of variants in SHSY5Y cells demonstrated an increase in NMDA induced excitotoxicity in contrast to wildtype SCYL2, likely via anti-oxidation pathways; and iii) In the

fourth family, a rare variant in PDGFB was identified as a likely cause of primary familial brain calcification. Besides referral and diagnostic value of these rare/novel disease causal gene/variant findings, enhanced understanding of disease biology and paving way for new therapeutics, such as SCYL2 as an inhibitor of excitotoxicity, seem likely outcomes.

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09. VERMA (Rashmi)
Genetics Dissection of Resistance against Sclerotinia Stem Rot (SSR) and Development of Transgenics for Improved Resistance in *Brassica Juncea.*
Supervisor : Prof. Jagreet Kaur
Th 25591

Abstract
(Verified)

Sclerotinia Stem rot (SSR) caused by the necrotrophic fungus Sclerotinia sclerotiorum (Lib.) de Bary is emerging as a serious disease of Brassica juncea, a major oilseed crop of the Indian subcontinent. An effective control of the pathogen is greatly limited due to the lack of strong resistance available in the cultivated varieties as well as the exotic germplasm. In the first part of this study, I have carried out screening of some Brassica germplasm to identify tolerant and susceptible genotypes. East European line, Donskaja-IV (Don-IV), showed significant tolerance against SSR. A double haploid (DH) mapping population derived from TM-4 and Don-IV (TD-DH) comprising of 94 lines was assayed for SSR resistance under field conditions using stem inoculation procedure. QTL analysis revealed that the resistance to SSR is governed by several minor QTLs and is influenced by the environment. I identified 8 QTLs for SSR resistance of which only one QTL qSSRA10-1 (2.6 Mb), was identified reproducibly in two consecutive growing seasons. qSSRA10-1 with an R2 of 16.97, explained 18.77% of the phenotypic variance against SSR. Furthermore, qSSRA10-1 spanning region has several genes related to defense responses like-protein kinases (serine/threonine), WRKY transcription factor, oxidation-reduction process-related genes, and Disease resistance protein (TIR-NBS-LRR) making it a promising QTL for fine mapping and functional validation. The second part of the thesis elaborates on the genetic engineering of B. juncea for enhanced tolerance against SSR. Three candidate genes Barley Oxalate Oxidase (BOxO), At Polygalacturonase Inhibitor Proteins (AtPGIP), and At Non-expressor of PR1 (NPR1) were expressed independently under a strong constitutive 35S promoter in B. juncea. Expression of BOxO and AtNPR1 provides improved tolerance against SSR over multiple generations (T4). It will be worthwhile to test if pyramiding the two transgenes can further improve the resistance in the highly susceptible variety Varuna.

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