

Current Research in Secondary Metabolism of Medicinal Plants in India

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Introduction:

My article is focused on my studies on “Interrelationship between gene silencing phenomena in *Drosophila melanogaster*” as well as my current interest on secondary metabolism in medicinal plants. Gene silencing refers to reduction or ‘knock down’ of a particular gene. It can operate at transcriptional or post transcriptional level. At post transcriptional level double stranded RNA acts as guide for sequence specific cleavage of homologous mRNA transcripts or for translational repression. At transcriptional level binding of multiprotein complexes of Polycomb (PC) or Heterochromatin Protein 1 silence the expression of a gene, by obstructing access to transcription factors. My study has attempted to establish a link between various such silencing processes.

I have been instrumental partly in establishing the role of RNAi machinery in heterochromatin formation at tandem repeats of drosophila eye color reporter gene *white*. This was first study in a metazoan demonstrating the role of RNA in transcriptional silencing. This research has been published in esteemed journal Science.

Using *en-w* transgenes - carrying noncoding *engrailed* polycomb response element fused to *white* coding sequence in a hybrid transgene - I have tried to establish a link between co-suppression of dispersed transgenes and pairing sensitive repression (PSR). Mutations of the minimal 181bp *en* PRE were analyzed for their ability to show PSR and co-suppression. Thus sequence determinants of the two independent silencing pathways were determined. Previous reports had indicated the requirement of RNA silencing factors for PC mediated transcriptional gene silencing (TGS) of *Fab7* PRE. However in my studies of *en-w* transgenes the interplay

between RNA silencing and TGS seems to be different. The transcriptional repression of *en-w* transgenes is enhanced in the mutants of RNAi pathway. Thus these studies indicate how various polycomb response elements previously thought to be behaving similarly can show subtle differences.

Finally, I have characterized a novel gene *Gas41*, whose loss of function mutations disrupt RNA silencing of *white*, the drosophila eye color gene in sensor transgenics *encoding white* hairpin RNA. Loss of silencing in microRNA sensors & reduction of various mature miRNAs in *Gas41* mutants, suggests a role of *Gas41* in microRNA biogenesis. Moreover, it also affects heterochromatic silencing & assembly of its components and position effect variegation in *w^{m4h}* & *Sb^v*. This is corroborated by loss of Histone 3 Lysine 9 methylation mark & reduction in Heterochromatin protein 1 binding. These results demonstrate the crucial role *Gas41* plays in bridging the transcriptional and chromatin silencing pathways in the nucleus. This important finding offers insights towards establishment of a coherent link between the two silencing processes.

In addition, using bioinformatics tools we have predicted miRNA genes in avian influenza and adenoviral genomes. The predicted targets of these microRNA in host human genome identified a wide spectrum of genes including genes involved in signal transduction pathways, transcriptional factor etc. Most importantly genes involved in RNAi and chromatin remodeling pathway were also the part of predicted targets. This proposed an alternate host-pathogen relationship and complexity in which miRNA plays an important role and unravels a new mechanism how viruses invade and thrive in the host environment.

Current Research:

A variety of organic compounds are synthesized by plants that are chiefly classified as primary and secondary metabolites. Primary metabolites are required for basic processes like photosynthesis, respiration, growth and development. Secondary metabolites are other phytochemicals which are accumulated in unusually high amount – not as mere intermediates of chemical processes. These compounds are very diverse and their distribution is often limited to

related species. Plant Secondary metabolites are the major source of novel compounds, and about 60% all FDA approved drugs are either natural products or their derivatives. Taxol and Artemisinin are examples of a few blockbuster drugs that are natural products.

My lab currently works on elucidating the secondary metabolic pathways in medicinal plants, with a long term objective of producing some of these metabolites in simpler organisms such as yeast. Functions of many such secondary metabolites are not well established. We are also trying to understand the function of these metabolites in plants. We employ both genetic as well as molecular approach to find the genes that either encode the enzymes of secondary metabolism pathway or that have regulatory roles pertaining to expression of secondary metabolites. *Coleus forskohli* is a medicinal herb that produces a labdane diterpene named forskolin. Forskolin is a reversible adenylate cyclase activator and is widely used in cell biology assays. Its unique chemical structure with eight chiral centres has intrigued synthetic chemists for a long time, and the chemical synthesis process is very lengthy and expensive.

The plant remains the only viable source of forskolin. Recently, 1,9 dideoxy forskolin, a forskolin synthesis intermediate was shown to have potent anti-cancer activity. Despite the discovery of forskolin about three decades ago, there is almost no information available on the genes involved in the biochemical pathway that produces forskolin. Forskolin is known to be synthesized in the leaves and then it gets transported to roots, where it gets stored. This provides opportunity to make subtractive EST libraries in order to identify genes involved in synthesis of this compound. We are preparing and sequencing the subtractive EST libraries from *Coleus forskohlii*. Detailed annotation will be performed, followed by full-length cloning and heterologous expression of candidate genes. Their activities will be confirmed through *in-vitro* assays, as well as through RNAi mediated knockouts, *in-vivo*. We also intend to perform genome wide mutagenesis screens to identify these genes.

Another metabolite Rohitukine is biosynthesized in a few tree species of Meliaceae family, such as *Amoora rohituka* and *Dysoxylum binectariferum*. Structure activity studies with this metabolite led to the development of US-FDA approved anti-cancer drug Flavopiridol, a potent and specific inhibitor of human Cyclin Dependent Kinases (CDK). Here also we are interested in

identifying the genes involved in biosynthesis of rohitukine as well as to try and understand the function of rohitukine in the plants.

Profile:



A Gold medalist in Bachelors and Doctorate in Molecular Biology and Genetics from a premier research institute of India, the Centre for Cellular and Molecular Biology. Dr. Gandhi, has been awarded Research Fellowships from CSIR, and has won various state level science awards. His doctoral research focused on understanding the interrelationships between various gene silencing phenomena using the *Drosophila* model.

Author's present research focuses on understanding various aspects of secondary metabolism in medicinal and aromatic plants at the prestigious Indian Institute of Integrative Medicine (IIIM) CSIR, Jammu India.
