CHAPTER 28

GENETICS

Doctoral Theses

01. BM. MINHAJUDDIN
Studies on the tapetum-specific promoter TA29 from tobacco: (i) cis-element identification by Linker Scanning Mutagenesis and (ii) challenges in transcription factor analysis.
Supervisor: Prof. Pradeep Kumar Burma
Th 28794

Abstract

The tapetum-specific TA29 promoter from Nicotiana tabacum, identified in 1990, has been widely exploited in plant biotechnology, particularly in combination with the barnase–barstar system for generating male sterility–restorer lines in hybrid seed production. Despite its extensive application, relatively little is known about the transcriptional regulation of TA29 at the cis- and trans-acting levels. Previous work from our laboratory showed that when TA29 promoter–reporter constructs were introduced into tobacco, a small percentage of independent transgenic lines exhibited reporter gene expression at ectopic locations. While ectopic expression due to position effects is not uncommon in transgenesis, in this case the expression was consistently restricted to meristematic regions such as the root tip and axillary meristems. To the best of our knowledge, such spatially confined 'leaky expression' has not been reported earlier to the report from the lab, for any plant promoter. The first part of this thesis thus investigates the basis of leaky expression by dissecting the TA29 upstream regulatory module (promoter and 5′ UTR) using Linker Scanning Mutagenesis. This unbiased strategy identified a key cis-element, CTTG–N_5–CAAG, located downstream of the putative TATA box. This motif is the binding site for NAC050, which recruits JMJ14, a histone H3K4 demethylase, known to act as a negative regulator of gene expression through chromatin modification. Based on these findings, we propose that the observed leaky expression arises from the combined influence of this cis-element and the genomic context of the inserted transgene. The second part of the thesis focuses on expressing TA29-interacting putative transcription factors in E. coli for DNA-binding assays. Since most full-length transcription factors accumulated in insoluble fractions, multiple strategies were evaluated, of which expression of DNA-binding domains proved most successful in obtaining soluble, purifiable recombinant proteins suitable for downstream functional analyses.

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02. RITIKA KAPILA
Functional insights into MTG3 mediated mitochondrial ribosome biogenesis and translation initiation link in Saccharomyces cerevisiae.
Supervisor: Dr. Kaustuv Datta
Th 28793

Abstract

Ribosome biogenesis is a complex and highly regulated multistep process aided by numerous energy-consuming auxiliary factors. GTPases form the largest class of auxiliary factors used by bacterial, cytosolic, and mitochondrial ribosomes for their maturation. Mtg3, a circularly permuted YqeH family of GTPase is implicated as an auxiliary factor for the biogenesis of the mitoribosome small subunit. However, its precise mechanistic role has not been fully characterized. Mtg3 is likely to bind to precursor mitoribosome molecules in vivo, however, this interaction has not been observed in mitoribosome extracts. In my study I have delineated the specific conditions necessary for preserving the association of Mtg3 with mitoribosomes upon extraction from mitochondrial membranes and separation on a sucrose density gradient. I have shown that the C-terminal domain of Mtg3 is required for robust binding to the mitoribosome and the N-terminus supports this attachment site as depicted by the sucrose gradient analysis. Ribosome profiling of the wildtype and Δmtg3 cells does not alter the relative ratio of small to large subunit. However, the translation is decreased in mtg3ts cells, suggesting either defective ribosome subunit formation or the subunits are formed but remains inactive. Furthermore, point mutants likely to abrogate GTP/GDP binding and GTPase activity compromise the protein`s function in vivo. Surprisingly, the association with the mitoribosome was not compromised in mutants likely to be deficient for nucleotide binding/hydrolysis Furthermore, Mtg3 has been shown to associate with mRNA molecules via the nucleic acid binding domain at its N-terminus. To investigate the role of Mtg3 in translation initiation, mRNA loading on mitoribosomes were examined in wild type or mtg3 CKRC94-97AKAA cells. It was observed that mRNA loaded onto mitoribosome polysomes were severely diminished in mtg3 CKRC94-97AKAA mutant in comparison to wild type. Thus, my finding supports a model wherein Mtg3 binds to a precursor mitoribosome through its C-terminus, to facilitate a conformational change or validate a folding intermediate driven by the hydrolysed energy from GTP/GDP, required for loading of mRNAs on the mitoribosomes.

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03. SHINGNAISUI (Khanchuila)
Functional Characterisation of Small Parvulin, Pin4, in Dictyostelium discoideum.
Supervisor: Dr. Aruna Naorem
Th 28302

Abstract

Parvulin PPIases accelerate cis/trans isomerization of phosphorylated or non-phosphorylated Ser/Thr-Pro moieties in target proteins and regulate cell proliferation, gene expression, etc. Dictyostelium discoideum exists as unicellular

amoebae feeding on bacteria and respond to starvation by aggregation of amoebae into multicellular fruiting bodies. It encodes two parvulins, PinA, a homolog of Pin1/Ess1, and Pin4, a second parvulin showing high conservation with human Par14 which plays diverse roles in transcription, ribosome biosynthesis, chromatin remodeling, etc. Hence, considering its distinctive life cycle and conservation of genes involved in various signaling pathways with humans, we attempt to elucidate the role of Pin4 in cell growth, development and cellular differentiation in D. discoideum. mRNA expression analyses revealed that pin4 is expressed throughout growth and development but is spatially expressed in the prestalk/stalk cells. Subcellular localization studies found that Pin4 is predominantly observed in the cytoplasm. Wild-type Ax2 cells overexpressing Pin4 and its mutants exhibited growth and developmental phenotypes such as delayed aggregation, slug arrest, and aberrant fruiting bodies having defective spores with short or no stalk. Rescue experiments with wild-type cells could partially restore the developmental defects of Pin4 and its mutants to normal, suggesting the involvement of diffusible molecules and inherent factors. Further analysis of these cell lines in growth and development indicates that Pin4 may regulate phagocytosis, cell motility, chemotaxis to cAMP, and cell interaction with its surroundings. Moreover, gene expression profiling in the aggregation process reveals a significant alteration in the expression of cAMP signaling components and cell adhesion molecules in Pin4 and its mutants overexpressing cells. Our studies provide evidence for a novel role of Pin4 in normal growth and development of D. discoideum independent of PinA expanding the importance of parvulin PPIases in cell movement and cell differentiation.

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04. SHIVAM NANDA

Functional characterization of a mediator complex protein Med21 of Dictyostelium discoideum and the evaluation of this protist as a host to express Plasmodium falciparum genes.

Supervisor: Dr. Aruna Naorem

Th 28792

Abstract

The Mediator complex is a conserved multiprotein assembly that relays regulatory signals from gene-specific transcription factors to RNA polymerase II in eukaryotic transcription. Studies in different model organisms found that perturbation of its individual subunits shows diverse phenotypes and is even implicated in human diseases. This work was initiated in Dictyostelium discoideum to understand the function of one of its subunits, Med21, in growth, starvation induced development, cell patterning and cell differentiation using bioinformatics and genetic studies. Computational analysis revealed significant conservation of Med21 with its yeast and human orthologs with a distinct glutamine and proline homopolymers. RT-PCR found that med21 is expressed during growth and development with a peak during aggregation. Subcellular localization studies showed Med21 is predominantly nuclear in growth. Overexpression of med21 led to larger cells with pinocytosis defects. These cells also formed fewer and smaller developmental structures, abnormal fruiting body with round defective spores. Cells overexpressing med21 formed slugs with reduced prestalk/prespore ratios and sorted preferentially to the prestalk region when mixed with wild-type cells. Cells lacking med21 grew normally but exhibited severe

developmental defects like excessive stream breakup, small fruiting bodies with defective stalks and irregular but viable spores. Further, comparative genomics between D. discoideum and Plasmodium falciparum revealed conserved and unique genes with most post-translational modulators being conserved to facilitate protein expression indicating D. discoideum may serve as a host for expressing P. falciparum genes. Further, a protein containing Ankyrin repeats from P. falciparum was successfully expressed in D. discoideum establishing it as a suitable host. Collectively, the work in this thesis highlights Med21 as a key regulator of growth, development, and morphogenesis in D. discoideum and also establishes D. discoideum as a host for expressing P. falciparum proteins with repeats, enabling functional studies for drug and vaccine development against Malaria.

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