Title: Deciphering Functions & Interactions of Fission Yeast Splicing Factor SpSlu7 Relevant to Constitutive & Alternative Splicing.

PhD THESIS ABSTRACT by TitashSen (S.R. No. 03-04-00-10-11-11-1-08605)
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The fission yeast genome with abundant multi-intronic transcripts, degenerate splice signals and presence of alternative splicing machinery is an attractive unicellular fungal model to investigate splice-site recognition and assemblymechanisms relevant to other fungal, worm, fly and higher eukaryotic genomes.Earlier work in the laboratory showed fission yeast SpSlu7 (homolog of budding yeast Slu7, Synergistic Lethal with U5-snRNA) has pre-catalytic intron context dependent splicing functions contrasting the predominant second step splicing role of budding yeast Slu7. Here we have investigated partners of SpSlu7 to discover its role splicing and other regulatory processes.

Part I: Understanding SpSlu7 functional interactome by genetic and biochemical approaches

We took up investigations todelineate the functional interactome of SpSlu7 that would mechanistically explain the pre-catalytic splicing arrest of *spslu7-2* cells.Affinity purification of epitope tagged Slu7 associated complexes was donefollowed by mass spectrometry. The proteins associated with SpSlu7indicate its presence inboth pre-catalytic and second step spliceosomes. Genetic interaction assays were then carried out to validate and interpret these physical associations. Double mutants of *slu7-2* with other splicing factor mutants, acting at distinct stages of the splicing pathway, were generated and studied. Taken together, we deduce an early pre-catalytic recruitment of SpSlu7 and a continued association afterthe first catalytic reaction. Further, in cells metabolically depleted of SpSlu7-2 the intron lariat intermediateRNA species was detected for intron 1 in transcript *tfIID*. This is a signature of poor second step splicing and thus demonstratesSpSlu7+functions improve second step splicing. Notably, other data from proteomic and genetic interaction studies showfunctional SpSlu7association with components of transcription, gene silencing and RNA decay machineries. Additionally the data uncovered a novel non-canonical Slu7function in meiotic RNA elimination in mitotically growing cells. In a parallel genetic screen for suppressorsof the cold sensitive *slu7-2* therevertant *UV10*was obtained. Careful assessment of growth and cellular phenotypesconfirmed thatsuppression in *UV10* was interactional and hinted an unanticipated effects on cellular pathways of mating and cell septation. Whole genome re-sequencing has been employed to identify candidate for the suppressor.

Part II: Probing physiological relevance and mechanistic insights into SpSlu7 dependent fission yeast alternative splice events.

The second part of the study probed possible physiological relevance and mechanistic insights of certain fission yeast exon skipping, intron retention and alternative splice site selection events and their dependence on SpSlu7. We identifiedstress and growth condition specific splice isoforms for certain transcripts.By assessment of the combinatorial effects of the intronic *cis* features, transcription elongation kinetics and splicing factor SpSlu7 activity, we deduced that suboptimal splice signals were the primary determinants of the low frequency usage of alternative splice sites. Additionally, we inferred use of certain alternative splice sites is co-transcriptional. Interestingly under conditions of mild thermal stress while SpSlu7 nuclear localization was unchanged, a distinct puncta like nuclear aggregation was noted foran interactor- branchpoint recognition protein U2AF59. These data underscorethe likelihood of stress-mediated remodelling of splicing factor complexes in fission yeast akin to higher eukaryotes.Overall, this study derived important insights

on spliceosomal associations of splicing factor SpSlu7 and elucidated functions in splicing, meiotic RNA elimination, and stress-specific alternative splicing.