JAMES RENNIE REQUEST REPORT
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I would like to begin my report by thanking the James Rennie Request fund for providing me with the opportunity for attending the RNA Society Meeting, July 2000 held in Madison, Wisconsin. The five day conference provided me with a stimulating environment and I gained some very useful feedback and advice on how to complete my PhD studies in my final year. Notably, I was able to network with fellow scientists who after the meeting kindly provided me with materials to further my investigations into the characterisation of the nuclear cap-binding complex (CBC). In addition, the meeting allowed me to view my work in context of the RNA world and so appreciate my contribution in this evolving field. This report provides a summary of the specific feedback I received from my poster presentation and the subsequent experiments I performed as a direct result of attending this meeting.

RNAs transcribed by RNA Polymerase II are characterised by a monomethylguanosine cap. The cap is implicated in various aspects of RNA metabolism. In the nucleus, the cap is involved in RNA stability, pre-mRNA splicing, 3' end processing, RNA export and nucleolar localisation of some small nucleolar RNA. In the cytoplasm, the cap is required for cap dependent translation. The stimulatory effects of the cap in pre-mRNA splicing, 3' end formation and U snRNA export are mediated by the nuclear cap binding complex (CBC). CBC is a heterodimer comprised of cap binding proteins CBP20 and CBP80. Homologues of both subunits have been identified in a variety of eukaryotes and CBC function in pre-mRNA splicing is conserved from yeast to humans.

Although CBC functions in three diverse aspects of nuclear RNA metabolism, little is known about the proteins CBC interacts with or the mechanisms by which CBC facilitates these processes. To further characterise the role of CBC in nuclear RNA processing, we are using the genetic and biochemical tools available in *Drosophila* research. The GAL4/UAS system is being used to identify proteins that mediate CBC function. A series of carboxyl terminal (C-T) truncations in CBP20 and CBP80 were generated under UAS control and transformed into flies. The CBP20/CBP80 transgenic lines were crossed with various GAL4 drivers and the progeny screened for dominant phenotypes. Dominant truncations have been identified in both CBP20 and CBP80. We are currently characterising the CBP80 C-T dominant mutant. Preliminary results from genetic screens have identified 7 deficiency mapped regions on the

2nd and 3rd chromosomes which modify the CBP80 C-T dominant phenotype. More excitingly, an allele of RNA Polymerase II large subunit which has a truncated CTD, also suppresses the dominant phenotype.

At the RNA society meeting, I was able to meet and discuss my results with post docs in Mark Mortins lab. Mark originally created the RNAP II fly strain that interacts with our CBC mutant. Afterwhich I was sent other flies mutant in the RNAP II 215 gene. This allowed me to test for further interactions with CBC and allowed me to conclude that CBC mutant does indeed specifically interact with the CTD of RNAP II. We were very excited about this result since the last few years has changed our perception of RNA processing. A key observation in the field of gene expression is that transcription, RNA processing and translation appeared to be mechanistically linked *in vivo*. This would make biological sense, as it would ensure the most efficient passage for gene expression. However, the molecular mechanisms by which these events are integrated remains to be elucidated. In the last few years, it has become increasingly apparent that RNAP II also contributes to the various RNA processing reactions required to synthesis a mature mRNA. In particular, the CTD of the largest subunit of RNAP II is found to integrate nuclear RNA processing with transcription. The CTD appears to not only recruit RNA processing factors to the sites of transcription but also directly functions in RNA cap formation, pre-mRNA splicing and RNA 3'-end formation. CBC also plays a key role in RNA nuclear metabolism, therefore it may not be surprising that CBC also interacts with RNAP II. Daniel Morris whom I also met at the meeting, is very enthusiastic to progress with this work and perform far westerns to determine whether CBC and RNAP II CTD interact directly/ indirectly. As a result, I am currently generating recombinant CBP20 and CBP80 proteins which I hope to send to him soon. We are currently writing a paper that describes this and other *Drosophila* work that I have performed during my PhD. Finally, I would like to thank the committee again for generously granting me travel money to attend the RNA meeting and subsequent opportunities that followed.

Thankyou

Yours Sincerely,

Lubna Ataf