

P023 The intergenic spacer of the *Drosophila Adh-Adhr* dicistronic mRNA stimulates internal translation initiation
Preethi Ramanathan, Jiannan Guo, Rebekah N. Whitehead and Saverio Brogna

*University of Birmingham, School of Biosciences,
Edgbaston, Birmingham, B15 2TT, UK*

The eukaryotic ribosome normally cannot be recruited upstream of internal ORFs and therefore polycistronic mRNAs are not efficiently translated in eukaryotic cells. However, examples of dicistronic mRNAs have been reported in *Drosophila* and other eukaryotes, and it was proposed that the intergenic spacers might contain internal ribosome entry site (IRES) elements. Here we investigated the translation mechanism of the dicistronic *Adh-Adhr* mRNA of *Drosophila melanogaster*. To test whether the intercistronic spacer mediates internal translation initiation, we constructed dicistronic reporters by replacing the *Adhr* coding region with firefly luciferase; and expressed in S2 cells. Additionally we generated constructs in which the intergenic spacer was inserted between the GFP coding region and the *white* gene of *D. melanogaster*, and placed it under control of an eye specific transcriptional promoter. Our results indicate that the full-length intergenic spacer in fact stimulate translation of the internal ORF, both in S2 cells and transgenic flies; and that this sequence is significantly more efficient in promoting translation initiation than the previously described IRES in the 5' UTR of the *Ultrabithorax* gene. Interestingly, we observed that transcripts derived from intron-containing constructs give higher translation yields, suggesting a previously unreported link between pre-mRNA splicing and efficient internal translation initiation.