

P018 Tetra-cysteine tagging of RNA decay pathway components
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Regulation of gene expression involves many post-transcriptional events with specific proteins acting to facilitate or repress translational processing of RNA transcripts. The location of such proteins and subsequently the sites within the cell that they interact with and, in some cases, sequester RNA gives valuable insights into protein function and organelle composition. To date, several proteins required for fundamentally important RNA-associated processes, such as members of the RNA decay pathway, have been analysed by fusion with Green Fluorescent Protein (GFP) or one of its derivatives. Recently a new method of tagging has been devised whereby the gene of interest is 'tagged' with a tetra-cysteine motif (Cys-Cys-Pro-Gly-Cys-Cys) that preferentially binds biarsenical cell-permeable dyes (FIAsH-EDT₂ or ReAsH-EDT₂) which only fluoresce when bound the target protein tag. This method negates concerns about the validity of protein localisation when fused to a moiety as large as GFP (~26kD). In addition, we have integrated the tag into the chromosomal copy of the gene of interest thereby ensuring that gene expression is under the control of the endogeneous promoter thus avoiding any over-expression artefacts that can often arise when expressing tagged proteins from vectors. Using these technologies we have focussed on proteins involved in RNA-decapping, specifically Dcs1, Dcs2, Pat1 and Dhh1. Data from confocal microscopy studies of the localisation and distribution of these proteins and a comparison with published GFP studies will be presented.