Novel reporters for translational recoding by the foot-and-mouth disease virus 2A peptide

Jessica Colborn
Dr Jeremy Brown

Newcastle University

Institute for Cell and Molecular Bioscience

Introduction

The 2A peptide expressed in viruses such as foot-and-mouth disease virus (FMDV) is a commonly used and invaluable tool in biomedical technology. 2A is a small 'self-cleaving' peptide which when inserted into a protein coding sequence (gene) drives the production of two co-expressed proteins separated at the 2A site. In normal protein translation the elongating ribosome sequentially adds amino acids specified by the sequence of codons in the mRNA to a growing polypeptide chain via peptide bonds (Alberts, et al., 2008). However, when 2A is inserted into 'the middle' of or between genes protein translation is prematurely stopped at a glycine-proline pair within 2A, the first polypeptide is released and then the ribosome translates the second half, with, in effect, one peptide bond skipped. The 2 proteins are co-expressed in equimolar amounts.

As a result of its unique activity the 2A peptide has many applications in biomedical technology from cancer therapy (Li, et al., 2012) to neuroscience (Tang, et al., 2009), allowing co-expression of 2 or more proteins from a single ORF.

Aims

- Design novel reporters to measure the activity 2A within transformed yeast.
- Identify and analyse factors affecting the efficiency of the 2A driven ribosomal peptide bond skipping.

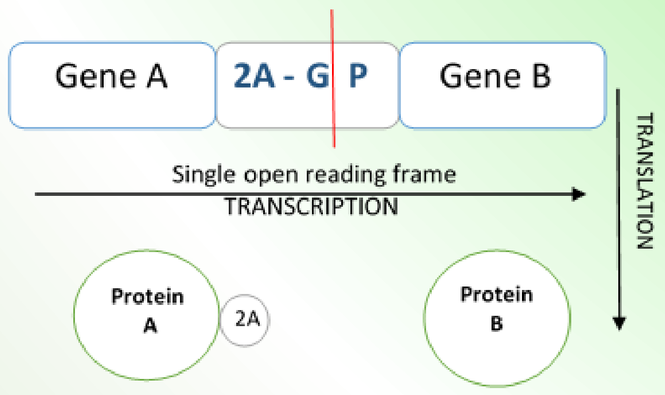

Figure 1. The mechanism known as ribosomal skipping takes place when 2A peptide sequence is cloned within/between genes. During protein translation the ribosome terminates translation between a glycine-proline pair within 2A releasing 'protein A' into the cytoplasm followed by the translation of 'protein B'.

Results

Nine plasmid constructs were designed and made, to test whether length, viral origin, or codon bias affected the efficiency of the 2A reaction. In each of these the 2A sequence (or a non-functional variant (2A*) was placed within GFP. Separation of GFP into 2 fragments leads to loss of fluorescence: 2A activity inversely correlates with fluorescence. Some constructs additionally contained RFP as a control for expression. Here analysis of a 19 amino acid FMDV 2A and 2A* is presented via several assays (fluorescence microscopy, Western blot and flow cytometry), which provided results consistent with FMDV being active within GFP, and the reporters as potentially useful tools for identifying conditions/mutations that affect the 2A reaction.

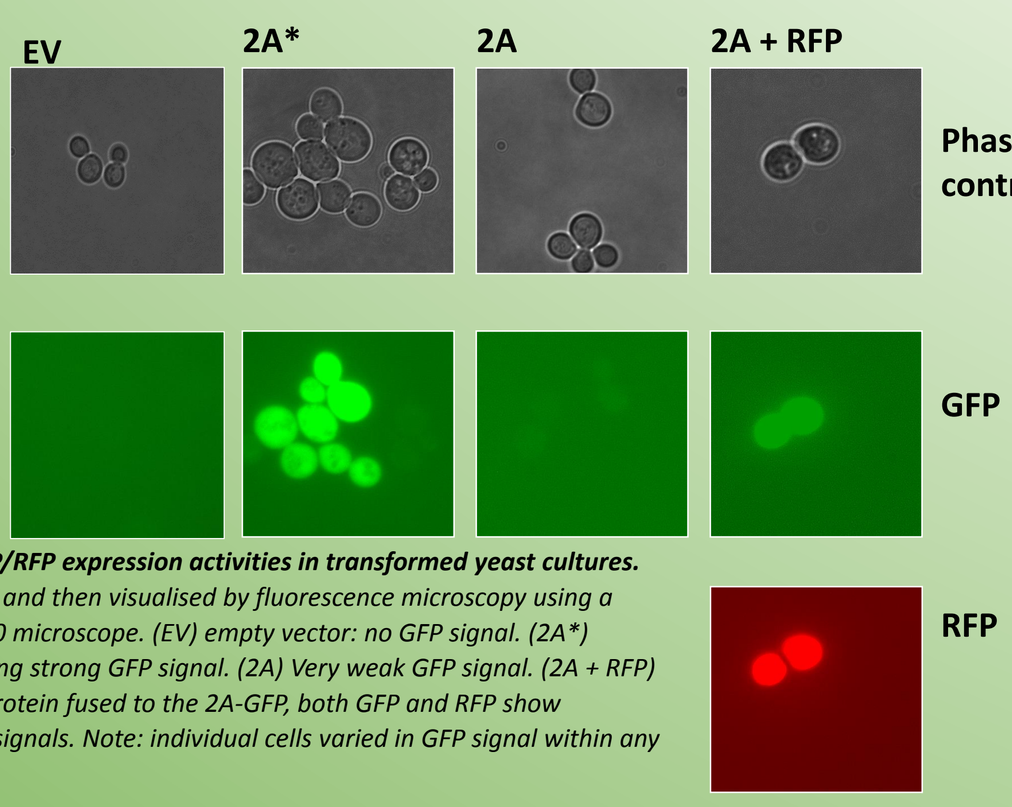

Figure 2. ***The GFP/RFP expression activities in transformed yeast cultures.*** *Cells were grown and then visualised by fluorescence microscopy using a Zeiss Axiovert 200 microscope. (EV) empty vector: no GFP signal. (2A*) mutant 2A showing strong GFP signal. (2A) Very weak GFP signal. (2A + RFP) red fluorescent protein fused to the 2A-GFP, both GFP and RFP show relatively strong signals. Note: individual cells varied in GFP signal within any one culture.*

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References

Alberts, B. et al., 2008. *Molecular Biology of The Cell.* 5th ed. New York: Garland Science .
Li, M. et al., 2012. 2A peptide-based, lentivirus-mediated anti-death receptor 5 chimeric antibody expression prevents tumor growth in nude mice.. *Molecular Therapy ,* 20(1), pp. 46-53.
Tang, W. et al., 2009. Faithful expression of multiple proteins via 2A-peptide self-processing: a versatile and reliable method for manipulating brain circuits.. *Journal of Neuroscience ,* 29(27), pp. 8621-9

Figure 3. ***Western blot of GFP expression.*** *Proteins were extracted from yeast cultures expressing the indicated proteins, resolved by SDS-PAGE, blotted to nitrocellulose membrane and proteins revealed using anti-GFP and anti-PGK antibodies. . Protein bands specific to GFP can be seen in lanes 2A* (mutant 2A + GFP); RFP (2A* + RFP).No bands for FP can be seen in lanes EV (empty vector) as a control, or 2A which supports the theory that GFP is inactivated by 2A. The bands of the same weight in all lanes are specific to the control gene PGK, a housekeeping gene which is constitutively expressed in all cells.*

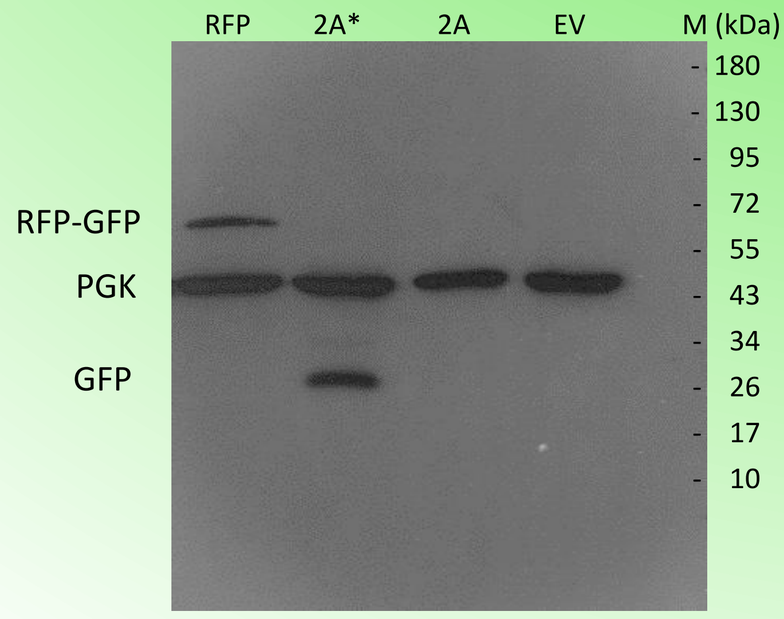
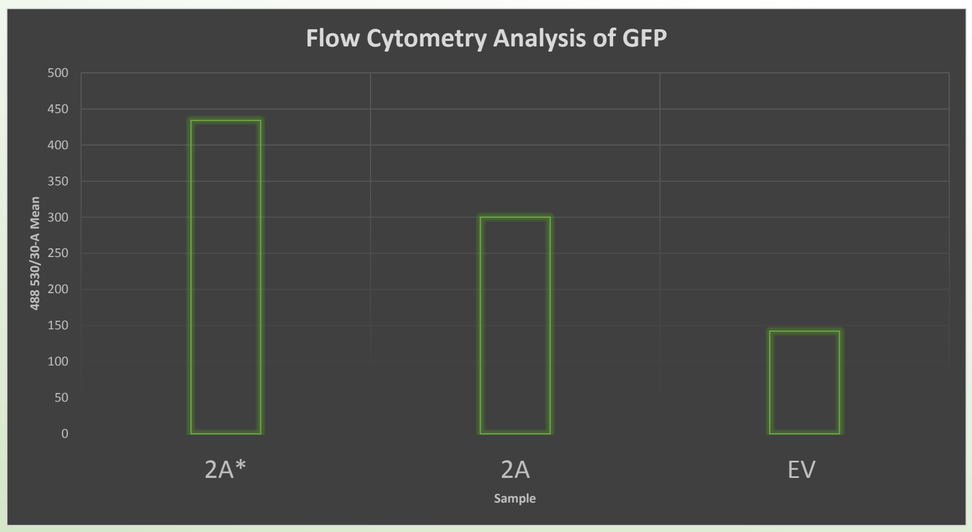


Figure 4. ***Flow cytometric analysis of cells containing GFP-2A constructs.*** *Yeast cultures were analysed for GFP expression. This was the first time flow cytometry had been used to analyse 2A activity, and the results show promise for future experiments. Data shown is the mean fluorescence intensity at 488nm with a 530/30 optical filter. (EV) empty vector : 142, this is a normal result for non fluorescently transformed yeast. (2A) Intact 2A: 300. (2A*) Mutant 2A: 432.*



Conclusions

- From the data shown here and other experiments we concluded that the FMDV 19 amino acid 2A had the highest cleavage efficiency compared to the 31 amino acid 2A and/or yeast optimised codons in the context of my project.
- Flow cytometry is a viable method to quantitatively and qualitatively measure GFP.
- Using FACS it should be possible to isolate populations of cells with varying efficiencies of 2A reaction..