A novel translational read-through event generates a sorting receptor in *Saccharomyces cerevisiae*

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Background: The yeast (*S. cerevisiae*) genome encodes three homologous sorting receptors Vps10p, Vth1p and Vth2p, that function in Golgi-vacuole transport. However a fourth protein of similar length can be generated from two adjacent reading frames, YNR066c and YNR065c, if the stop codon of YNR066c is read as sense and translation continues through the 24 nucleotides that separate the two ORFs (Figure 1). Western blotting (in a collaborator's laboratory) identified a protein of a size consistent with such a translation product. Stop codon read-through is frequently seen in viral, and occasionally in genomic transcripts, leading to synthesis of specific proteins or protein variants. Frequently this is reliant on secondary structures in the mRNA just following the stop codon that is read through. Examination of possible structures that could form after the stop codon of YNR066c, identified stem loop and bulge structure, Figure 2, which is hypothesised to be be required for this stop codon read-through event.

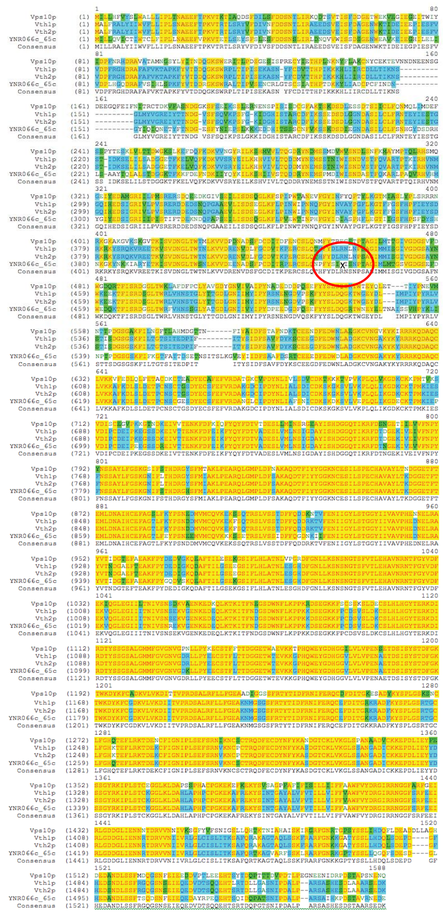

Figure 1: consensus sequence after gene alignment. (Doronina *et al*, (unpublished))

Aim of the Study:

- To identify how much of the stem loop structure needs to be present for the read-through of the stop codon to occur using constructs from previous experiments.
- To generate sufficient data to test the significance of the data.
- Use data to generate new constructs to further dissect requirements for the stop codon read-through event.
- Provide an independent test of read-through in a different system (in vitro translation).

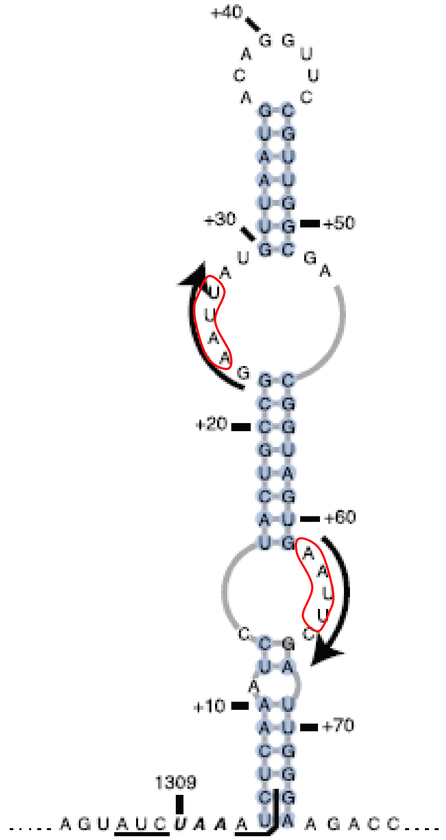

Figure 2: Diagram showing the predicted stem loop structure in the mRNA strand formed by base pairing. Areas circled in red highlight a possible fold and base pairing to form a possible pseudoknot structure. (Jeremy Brown, unpublished)

Methods:

Stop codon read-through was assessed using two reporters, LacZ and luciferase, present in frame and transcribed from the same promoter (figure 3). These were separated by fragments from the YNR066c-YNR065c junction, including the stop codon. The different constructs were expressed from plasmids in yeast, protein lysates were obtained from the cultures and the amount of luciferase relative to LacZ (and hence stop codon read-through) was assessed for each construct using a dual light assay (Applied Biosytems).

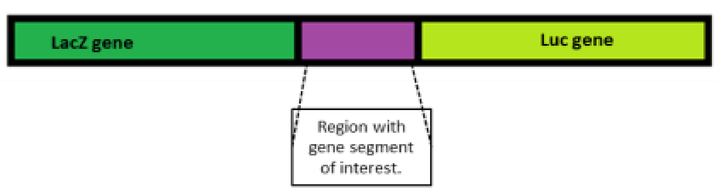

Figure 3: cartoon of plasmid with gene segment flanked by sequences used in the dual light assay.

Results:

- Previous experiment (Figure 4) indicated that the stem loop structure could drive efficient read-though of the stop codon in the lacZ-luc reporter, suggested that removal of parts of the stem-loop reduced read-through. Further, sequences upstream of the stop codon stimulated its read-through. This would be highly unusual, as stop codon read-through is not normally affected in this way
- Data obtained here confirmed these findings, though there was, in each set of data, considerable variation in the data obtained with the construct containing the complete stem-loop.
- Truncation of the loop (constructs 363 and 365) or removal of its 3' portion (364) reduced luciferase expression to >40%, indicating that most if not all of the stem loop is needed for 80-90% read-through of full structure with the stop codon

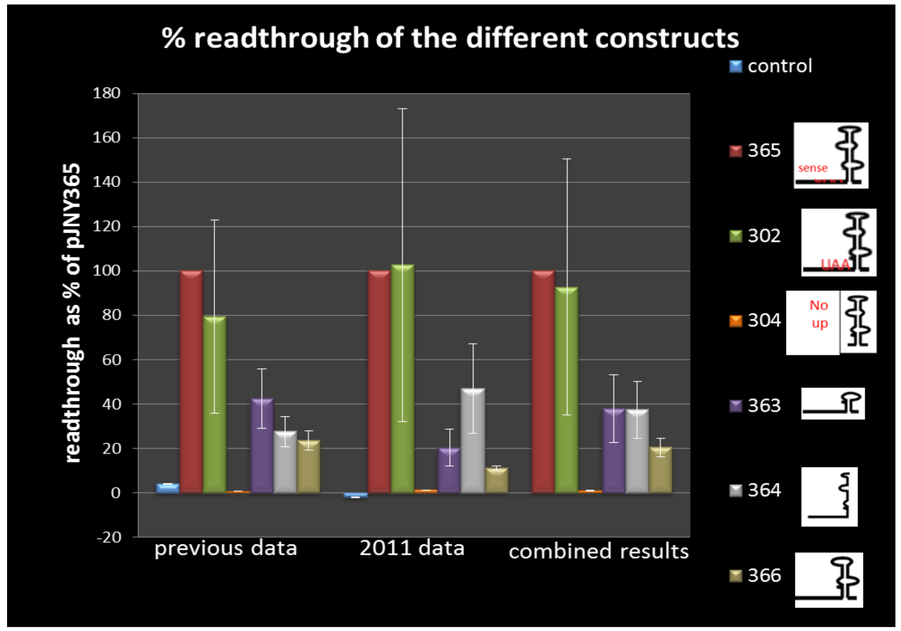

Figure 4: Graph shows the correlation between data collected in previous year experiments and the data collected in the 2011 project using the dual light assay. Variation is seen in the data but overall analysis shows that the method used and data collected in an experiment used over a number of years is reliable to enable reproducible data under the conditions of the experiment.

Conclusions

Conclusions: The project successfully generated a reproducible pool of data from the dual light assay experiment. New cloned constructs including one to disrupt the possible pseudoknot folding of the structure and one to test the possibility of read-through due to the repeated AUC codon before and after the stop which could cause the stop to be "jumped" have also been created for use with further experiments. A set of plasmids containing the same series of fragments from the YNR055c-YNR065c junction have also been made for in vitro translation, but time ran out before these could be tested.

Future Studies: There are a number of future experimentation that is needed to be sure of the relationship between the structure and the read-through event, including:

- Testing of new constructs such as one which prevents pseudoknot formation
- Testing the relevance of the repeated codon AUC before and after the stop signal as a possible "jump point" for the ribosome.
- Use the constructs in *in vitro* translation studies to see if the effect is the same as *in vivo* to eliminate possible contribution to the read-through event from the yeast cell.
- Testing if this read-through event is unique to *Saccharomyces cerrevisiae*
- Test the possible implications that virus mechanisms may use similar techniques during infection.

References:
Doronina Victoria A *et al*, (unpublished). An efficient stop codon read-through event generates a putative yeast sorting receptor.

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