



of a Biological Tool to Measure Intracellular pH *in vivo*

Introduction

- Nutrient-dependent changes in intracellular pH (pHi) affect the mammalian Target of Rapamycin (mTOR) signalling
→ ageing and age-related human diseases
- mTOR**: a serine/threonine protein kinase, which regulates cell growth, proliferation and survival, its activity can be measured using phospho-p70-S6 kinase antibody (p-p70S6K^{Thr398}) by western blotting
- E²GFP**: a ratiometric pH-sensitive green fluorescent protein (GFP) carrying 4 mutations (F64L/S65T/T203Y/L231H)

Aims

- Generate E²GFP to measure pHi in *Drosophila* S2 cells
- Test an effect of nutrients on mTOR activity in S2 cells

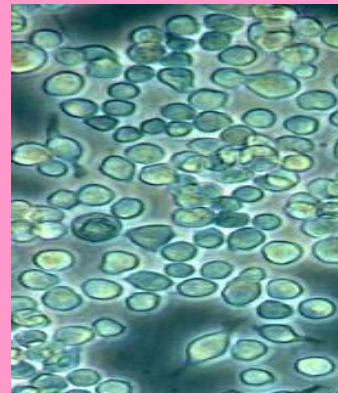
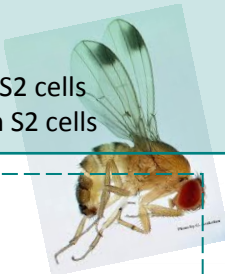


Fig.1 *Drosophila* S2 cells.

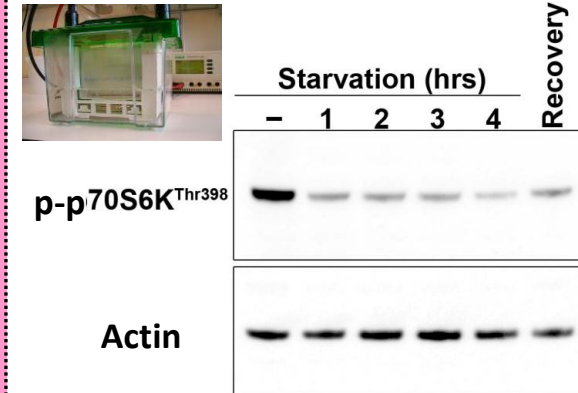


Fig.2 Images of western blot.

Methods

I. Site-directed mutagenesis-PCR:

pEGFP-NI (F64L/S65T)+Primers (T203Y,L231H)

Thermal Cycling

DpnI digestion

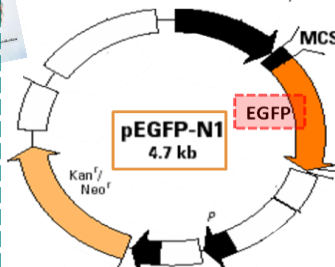
Transformation

agarose electrophoresis

sequencing

1st mutation

2nd mutation

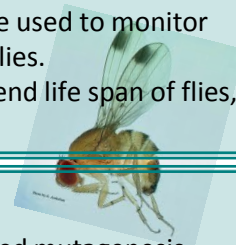


Results

- T203Y mutation of EGFP gene was achieved in the course of the studentship.
- Intensity of p-p70S6K^{Thr398} bands (activity of mTOR) relative to the loading control (actin) decrease with longer starvation and increase after recovery.

Discussion

- Final version of E²GFP probe is currently completed, which will be used to monitor changes in pHi and their relevance to mTOR activity and ageing in flies.
- Dietary restriction and reduction of mTOR activity, known to extend life span of flies, can be modelled using *Drosophila* S2 cells in tissue culture.



Conclusion

- pHi indicator, E²GFP was generated from pEGFP-NI by site-directed mutagenesis.
- Drosophila* S2 cell line model was validated as a tool to study the mTOR signalling.

II. Western blotting:

Treated S2 cells (starvation-0,1,2,3,4hrs;

1 hr recovery after 4 hr starvation)

→Bradford assay to measure protein concentration

→Western blot (p-p70S6K^{Thr398} vs actin as a control)

References

1. Bizzarri, R., et al., *Development of a novel GFP-based ratiometric excitation and emission pH indicator for intracellular studies*. Biophysical journal, 2006. 90(9): p. 3300-14.

2. Zoncu, R., A. Efeyan, and D.M. Sabatini, *mTOR: from growth signal integration to cancer, diabetes and ageing*. Nature reviews. Molecular cell biology, 2011. 12(1): p. 21-35.