Staff and students co-creating the curriculum, co-shaping the learning experience

Cristina Montiel-Duarte
Senior Lecturer in Biochemistry, CST

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BSc (H) Biological Sciences Biochemistry and Microbiology Pathway Leader, CST

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Cristina Montiel-Duarte
Undergraduate Research Project Module Leader, CST

How to take advantage of the undergraduate projects

- Involving students in shaping the learning experience
- Involving students in co-creating the curriculum

1. Shaping the learning experience

- Undergraduate project is individual

1. Shaping the learning experience

- Workshops

BIOL30001 Research Project module - November 2016

Workshops - a wish list

Session 1	Computer session	How to draw a graph on Excel and calculate SD and coefficient o… f statistis tests
Session 2	Computer session	Use of GraphPad for statistical analysis
Session 3	Computer session	Use of SPSS for statistical analysis
Session 4	Computer session	Use of Adobe Illustrator to prepare figures
Session 5	Lab session	How to use the balances and pH meters to prepare accurat…
Session 6	Lab session	Induction to Forensics labs
Session 7	Lab session	Use of Bunsen burner and aseptic techniques to p…are agar plates and perform… unts
Session 8	Lab session	Protein quantification, SDS PAGE and Western Blotting
Session 9	Lab session	Use of PCR machines and preparation/visualisation of DNA on an agarose gel
Session 10	Computer session	Quantification of bands obtained with the BioRad system
Session 11	Lab session	Use of cryostat for sectioning

Students input

If your tutees need to attend any of these sessions, please add their name, and N number (and Course if you know) below

NTU ID	Name	Course	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9	Session 10	Session 11

1. Shaping the learning experience

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NTU ONLINE WORKSPACE

BIOL30001: Research Project 201617 Full Year

Content | Dropbox | Edit Room | More | Student Dashboard | NTU Email | Timetable | My Tools | Help

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Lab information

Lab-related information

We have tried to put together in this section all the information that might be useful for those of you doing a lab-based project.

Sub sections:

- How to use some of the lab equipment
- Software for the lab
- General lab information: see the subunit 'lab-related' under the content.

If we have time, we will add more information to these sections. Also, we would appreciate your comments/help with videos for the equipment section. And overall, we hope you find it useful.

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1. Shaping the learning experience

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Table of Contents > Lab information > Equipment information > Common Equipment and how to use it

Common Equipment and how to use it

Lab equipment

What to find in this section

Although you would have been shown how to use the equipment in the lab, sometimes is useful to have access to that information again. A few students did kindly agree to provide a video of how to use some of the equipment we can find in our labs and you can get access to those videos here.

- How to use a 96-well plate reader.
- How to use a PCR machine.

(Note: if somebody else is happy to prepare more videos, please contact Dr Montiel-Duarte)

1. Shaping the learning experience

1. Shaping the learning experience

Students:

- Make decisions about the skills they need to develop
- Contribute with materials useful for their peers
  - Fostering creativity
  - Reinforcing understanding

2. Co-creating the curriculum

- Do you need to revamp material in any of your modules?
  - Go to www.menti.com and use code 93 02 15

… use the Research Project module and involve your students!

2. Co-creating the curriculum

- Example

NOTTINGHAM TRENT UNIVERSITY

Using Fluorescence Microscopy to Study GLUT4 Transporter Translocation

Matthew S. Critcher [1]

[1] Rosalind Franklin Building, Nottingham Trent University, Nottingham, UK

Introduction

GLUT4, a 54 kDa transmembrane protein coded for by *SLC2A4*, is a major glucose transporter involved in the process of insulin-dependent glucose regulation [1]. During periods of high blood glucose, insulin secretion by the pancreas leads to the translocation of the GLUT4 transporter from intracellular GSVs to the plasma membrane by poorly understood intracellular mechanisms. Since the discovery of GFP in 1955, fluorescence microscopy has become a valuable analytical technique. Once it was discovered that GFP could be expressed in other organisms, DNA technology became increasingly used alongside fluorescence microscopy for intracellular process determination [2].

The study aimed to use recombinant plasmids to produce GFP, and Snap-tagged GLUT4 transporter for transfection of the DU-145 cell line; and to study the intracellular location, and translocation of the GLUT4 transporter by using fluorescence microscopy.

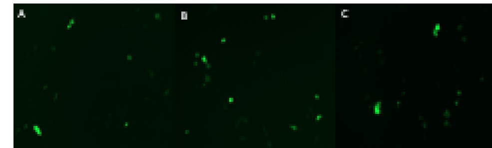


Figure 1: Transfection of the DU-145 cell line using the 2:1, 1:1 and 1:2 transfection conditions (x100 magnification). The 1:2 condition (image C) was chosen based on results seen in image C and other transfection attempts

Methods

The DU-145 cell line was cultured in DMEM 4.5g/L Glucose media containing 1% Penicillin/Streptomycin, 1% Glutamine and 10% FBS, and were left to grow in an incubator at 37°C, 5% CO_2 for 3-4 days until confluent. Once confluent, the cells were split down for re-seeding, or for use in analysis.

Using recombinant plasmids containing genes for GFP and Snap-tagged GLUT4, the translocation of GLUT4 transporters from the cytoplasm to the plasma membrane was monitored. The cells were initially treated with 3 transfection conditions using the GFP plasmid, to determine the best conditions (Figure 1). Once GFP had determined the desired transfection condition, the cells were transfected with a 1:2 DNA:PEI ratio using the GLUT4 plasmid. The GLUT4 transporter was expressed and then tracked using fluorescence microscopy, upon addition of the SNAP-Cell 505-Star substrate and insulin, to follow its translocation upon signaling by the insulin receptor.

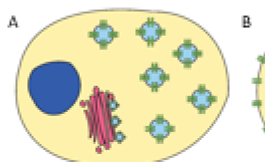


Figure 4: Expected results before and after the addition of insulin. (A) Cell in the absence of insulin. GLUT4 transporters present in GSVs in cytoplasm and trans-membrane of the Golgi. (B) Cell in the presence of insulin. GSVs fuse with plasma membrane implanting GLUT4 transporters in the membrane. (Original figure)

Figure 2: GLUT4- … addition of insu… fluorescence, (B)… Nucleus and GL… nuclei and GLUT4…

Figure 3: GLUT… magnification) at… seconds (D) from… well plate. Green… GLUT4 transporte…

Results

The DU-145 cell… DNA:PEI ratio u… be consistently… 1:1 and 2:1 rati… in transfected ce… ratio, was fou… membrane withi… to the wells. It… transporter wa… cytoplasm in t… insulin receptor.

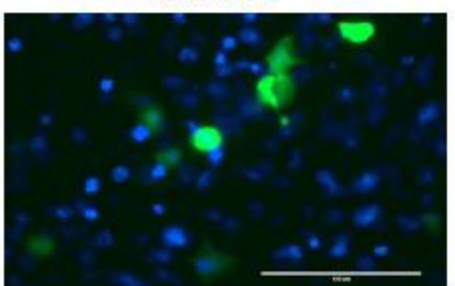
Conclusion

It can be concluded that the use of fluorescence microscopy is a valid and promising method for tracking the translocation of the GLUT4 transporter. Fluorescence microscopy has enabled the GLUT4 transporter to be tracked in real time after the addition of insulin, producing results similar to those

Table 1 Identifying optimal transfection conditions

PEI:DNA Ratio (µl:µg)	Cell Transfected (Yes/No)	Cell viability (%)	Comment
5:1	No	<80	No gene expression
4:1	Yes	<80	8 to 9 viable cells expressing both GFP and hIR-GFP genes per well
3:1	Yes	<70	1 to 2 cells per well expressing GFP with no hIR-GFP expression
2:1	No	<70	No gene expression
1:1	No	<70	No gene expression

hIR-GFP



Insulin receptor: a study of signal transduction

Jeffery Gyamerah[1,] and Cristina Montiel-Duarte[1]*

…d to develop a method for the …45 prostatic cancer cell lines … receptor – green fluorescent …sing polyethylenimine (PEI) in …ction of the human insulin … hIR-GFP expression was … using fluorescent microscopy … cells were transfected at a 4:1 …ncubated at 37°C for 4 hours. …bation period a phosphate-…) wash was performed and a …or 18 hours carried out. No …icking was observed, however, …reated with insulin.

…n receptor, INSR, Translocation, GFP, PEI

…TRODUCTION

…llitus is a chronic metabolic … incidence across the world, the …owing into an epidemic for a …here it is estimated that within … disorder will impact the ageing …creasing pressure on healthcare …ly in developing countries. …teristics of the disorder are: an …nsulin and impaired secretion of …, Obateru and Olokoba, 2012). A …ensitivity causes an increase in …ancreatic β-cells which is then …eased insulin clearance by the … al., 2003). At a molecular level, …ading to the increased hepatic …e internalisation of the insulin … measure of halting hormone … and Ussar, 2016). The opposite,

internalisation of the receptor is a mechanism aimed at sustaining signalling as, once internalised, the receptor has access to intracellular structures and proteins which partake in modulating the hormonal response (Sorkin and von Zastrow, 2002). The mechanisms underlying this trafficking, however, are still poorly understood (Morcavallo et al., 2014).

Insulin and the Insulin Receptor

Insulin is a peptide hormone found in blood which modulates carbohydrate metabolism by stimulating glucose uptake within skeletal muscle and adipose tissue. The hormone consists of 51 amino acids in two polypeptide chains, linked by three disulfide bonds (Soleymani et al., 2016). Biochemical and morphological studies focusing on insulin processing using fluorescent and autoradiographic labels were carried out as early as the 1980s, detailing insulin accumulation on the plasma membrane in clusters prior to rapid internalisation. This, coupled with the observation that hyperinsulinemia is common in both animal and human subjects, lead to the hypothesis that the receptor was also being endocytosed, metabolised intracellularly and, perhaps, recycled under normal physiological conditions (Olefsky et al., 1982).

Furthermore, employing radiolabels demonstrated the adsorptive endocytosis carried out by the IR, by covalently linking photoreactive /sup 125/I-N insulin to the receptor and observed its movement by employing electron microscopy, successfully showing the internalisation of both the hormone and its receptor (Gorden et al., 1982).

By 1987, the internalisation of the IR had been confirmed via a number of other IR-radiolabelled studies, one of which observed rapid internalisation of the IR in rat hepatoma cells (Fao). When the cells were treated with I125 insulin, by immunoprecipitating the IR with a tailored antibody, the researchers were able

2. Co-creating the curriculum

- Project findings applied to a new lab session in a year 2 module

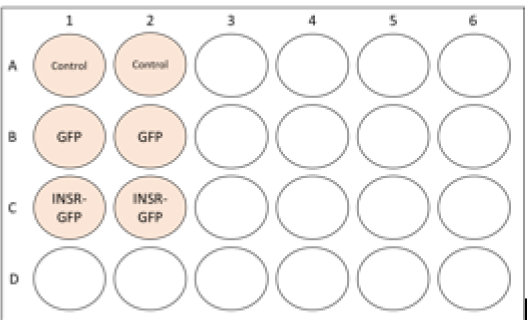
Visualising the Insulin Receptor (INSR)

Projected learning outcomes:

After this practical, you should know how to:

- Stain live cells nuclei
- Use a fluorescence microscope to visualise labelled proteins/DNA

Precautions before starting this practical: use gloves at all times and do not get in contact with the cells in the plate. The cells used in this practical are U2OS, human cells derived from an osteosarcoma, that have been genetically modified to express the Insulin Receptor attached to the green fluorescent protein (GFP). The cells have also been starved of serum, in order to see the effect of insulin treatment more clearly, similar to conditions described in Liu *et al.* (2016). http://www.nature.com/articles/srep35438.



Protocol:

1. Add one drop of NucBlue Live Cell Stain per well with cells.
2. Incubate your cells at room temperature for 20-30 min.

> During the incubation time, get familiar with how to operate the FLoid microscope by launching an interactive demo here.

3. From those wells (C1-2) with cells expressing the insulin receptor attached to the green fluorescent protein (INSR-GFP), take 200 µL of the media and transfer it to a 1.5 mL tube.
4. Add 30 µL of your insulin solution to the 200 µL of media and mix well.
5. Take your plate and the insulin-media solution to the EVOS FLoid microscope. Take a pipette and tips with you as well.
6. Visualise first the nuclei (blue) in your cells
7. Visualise now the transfected cells (green) – both the cells that only express GFP and the cells that only express INSR-GFP. Take pictures of these cells. Is there any difference in the protein distribution?
8. Add the 230 µL insulin-media solution to the cells that express the INSR-GFP and observe if there are any changes in the distribution of the insulin receptor. No need to wait for more than 3 min!

2. Co-creating the curriculum

- Project findings applied to a new lab session in a year 2 module
- Year 2 students get to:
  - Learn about transfections
  - Use the fluorescence microscope

Students as partners

- The Research Project module can be an useful source of material and opportunities to involve our students in shaping their own learning experience but also influence future students' learning.