

## Research Methods Session 3

### Critical review of a research article

This exercise is designed to introduce you to reading and interpreting the information contained in research publications. This type of publication will form the basis for much of your studies during the final year, both in taught options and your project, and it is important that you get in some practice at this.

Before Session 3 follow the guidance notes below, and work in groups to:

- **read and critically review the research article, and**
- **prepare to present and discuss the key points arising from the research article**

#### Introduction:

During your final year studies you will rely heavily on research articles to gather information. The context and necessary pre-requisite knowledge to make sense of these articles will invariably have been put in place before you come to read them, however it is not always straightforward to read and interpret this information. The research article accompanying this handout reports on a short study into the effects of the p21<sup>CIP1</sup> cdk inhibitor on cells. The context of this article should be already be apparent to you. Not only does the article link in with the p53 exercises that you have just undertaken, you will be familiar with the theoretical background to this protein, and the approach used, from the Cell Biology module, and in particular the practical you completed in December. With the exception of detail relating to inducible expression vectors, most of techniques should be familiar and / or straightforward, allowing you to focus on what the article reports and allowing you to gain experience of critiquing a research article. Appended to these guidance notes is a brief summary of all that you need to consider regarding the LacSwitch inducible expression system.

The p21 article is in fact adapted from a draft of a paper that was submitted for review by a specialist journal, and the authors and address have been changed. In this draft version the Results and Discussion section of the paper contains some flaws, and errors in interpretation of data that you might spot if you are being critical and analytical in your thinking (the error is quite fundamental and spotting it does not require specialist knowledge). Keep in mind that things are not always as they seem, so always look for accuracy and attention to detail.

Critically review this draft research article. In effect, you should put yourself in the place of the reviewer who would have been given the task of evaluating this work before acceptance for publication.

Use the questions listed below to structure your thinking think, and extract from it the key information, making notes along the way.

## Questions to answer:

### General

1. What is an abstract for? Is this a good Abstract (state your reasons and give it a mark out of 10)?
2. What is an Introduction supposed to do? Does the Introduction here succeed (state your reasons and give it a mark out of 10)?
3. Are the conclusions drawn by the authors justifiable and supported by the data (state your reasons and give the Conclusion a mark out of 10)?

### Methods

1. In what ways were the CHO cells engineered?
2. Why was it important to count viable cells (and would it have been useful to count dead cells too)?
3. Why was a horse radish peroxidase-conjugated secondary antibody used for Western blotting?
4. How do ELISAs work?
5. Why is propidium iodide used to measure DNA content?
6. How does Mito Tracker Green work?

### Results

1. What does Figure 1 show? Why is there variation between clones, and why are the differences in yield not so great as the differences in productivity in the presence of IPTG?
2. What does Figure 2 show? Why was a Western performed (rather than an ELISA), and what else would have been run on the gel (but is not shown in this figure)?
3. What does Figure 3 show? Why was it important to check the parameters measured in Figure 3?
4. What does Figure 4 show? Why was IPTG removed from some of the cells?
5. What does Figure 5 show? What effect does IPTG have on cell cycle progression?
6. What does Figure 6 show? Could the authors have used their measurements of cell diameter to derive a more accurate measure of cell size?
7. What does Figure 7 show? Is there a problem with the timing of this experiment?
8. What does Figure 8 show? How does this relate to data in other figures?

- keep your notes as brief as possible, concisely summarising thoughts and observations
- note down anything that you are unsure about or feel you cannot explain within the framework of your current knowledge
- keep in mind that for the most part you are not expected to know or read up on anything more than you have already covered in the course so far (although if there are gaps in your understanding this will be an obvious opportunity to consolidate your knowledge)

## Background: engineering cells for inducible expression of a protein: the LacSwitch system

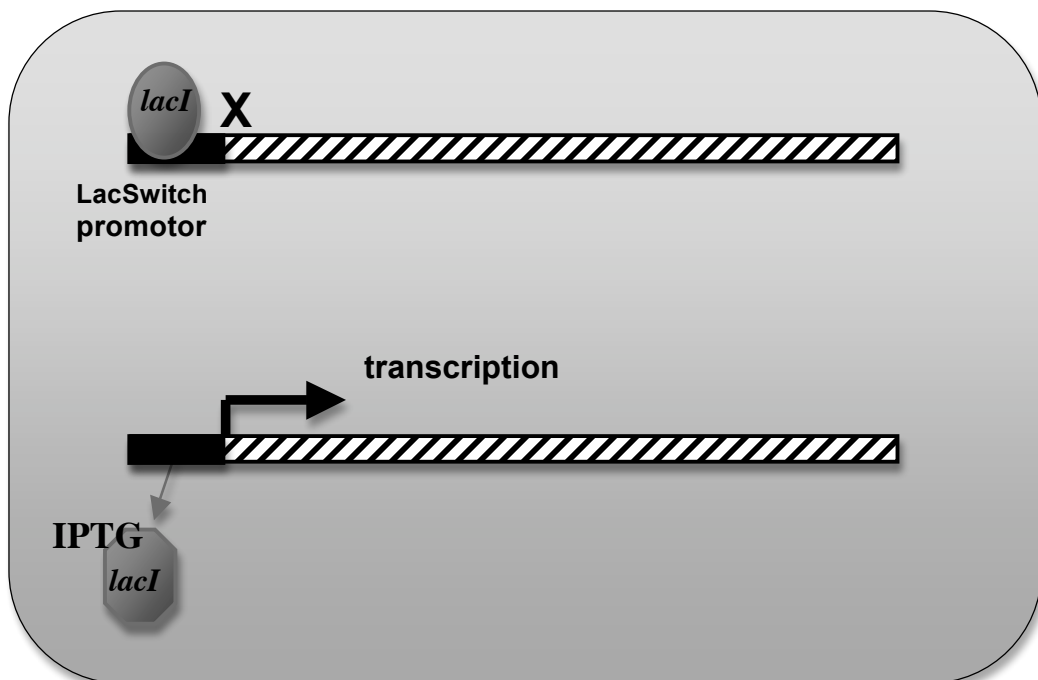
General principles:

You do not need to dwell on the “very techy” issues of what system is used, but be familiar with the key principles involved. Broadly these can be summarised as follows:

- all genes are controlled by promoters, that regulate the rate at which transcription occurs
- the objective (in this study) is to stably integrate a copy of an “exogenous” gene into the genome of cultured cells (by non-homologous integration)
  - think about why this needs to be non-homologous
  - think about expression of the “endogenous” gene
- expression of the “exogenous” gene needs to be under the control of an inducible promoter, that is coupled to the gene during construction of the recombinant DNA that will be introduced into cells
  - the gene is normally switched “off” and no mRNA protein expressed
  - the gene is switched “on” (expression induced) when required, by creating conditions which activate (or de-repress) the promoter associated with the “exogenous” gene
- promoters work by binding transcription factors and stabilising RNA polymerase in the transcription initiation complex

for the LacSwitch system:

- cells need to be engineered to express constitutively the *lacI* repressor protein that will bind to specific DNA sequences in the LacSwitch promoter
- the “exogenous” gene is coupled to the LacSwitch promoter and the recombinant DNA introduced into cells that have already been engineered to express *lacI*
- in these doubly transfected cells the LacSwitch promoter is normally repressed by the bound *lacI* repressor protein and no “exogenous” gene expression should occur if everything is working correctly (think about why it might not and what experiments you need to do to check)
- when IPTG is added to cells it binds to *lacI* protein and causes it to be released from the LacSwitch promoter, allowing transcription to occur (see diagram below)



## Tutor Notes

Each section of the paper should be reviewed, with the intention of identifying key points.

### 1. Abstract

a reasonable summary, although it echoes the flaws in the work

### 2. Introduction

in essence, the introduction gives background to metabolic engineering of cells (perhaps should not provide the main focus for discussion?), plus reasons for the work reported here and its main conclusions (focus?)

- Why is metabolic engineering being used?
- cell cycle arrest has previously been shown to result in increased productivity
- this study sets out to find out why, by investigating various cell / metabolic parameters
- results indicate growth is uncoupled from division, and that increased cell size is most likely reason for increased productivity

### 3. Methods

It is clear from the cell biology practical that many (a majority of) students are confused over the fundamentals of gene expression, transfection / stable integration of DNA, exogenous genes being expressed – constitutively or inducibly. I am sure that many still do not appreciate what gene expression is all about (some students were suggesting that they would extract DNA from cells to see which genes are expressed, and others are not clear in their thinking that changes in gene expression generally only involve a sub-set of genes. With this in mind:

- need to make sure that students understand the principles (if not the details) of engineering cells (transfection, selection)
  - the CHO-22H11 (parent cell line) already harbours an exogenous IgG gene (the “product” protein that is assayed to determine productivity / yield per cell)
  - CHO-3B2 cells have been derived by doubly transfecting CHO-22H11 with LacSwitch constructs (ie repressor and then inducible construct)
  - I have provided “key points” relating to LacSwitch – concepts not details are important
- visit why Western blotting used (many students when asked would choose ELISA to measure protein levels in cell lysates – says nothing about size of protein. Note that ELISA is used here on supernatants, so can contrast the two methods)
- visit cycle analysis – can they think of any other way to analyse cell cycle distribution? why RNase treat? (PI stains RNA as well as DNA)
- why use Trypan blue? (dye excluded from live cells, which are the only cells of interest here) – could be useful to also follow cell death – particularly during extended cultures, where cells are in stationary phase (lack of increase in cell numbers could reflect increased death as well as reduced proliferation – tie in with cycle analysis and reduced number of control cells S-phase)

### 4. Results and Discussion

Fig 1.

- cytostatic effect varies – probably due to differences in level of p21 induced
  - could have checked this, but not central to objectives
- product yield (B) is from fewer cells, hence productivity (C) i.e. amount per cell, is greater in +IPTG cells
  - this becomes clearer when you consider data from other experiments – fewer cells but larger, therefore amount produced per cell (productivity) is greater

Fig 2.

- NOTE equal numbers of cells were analysed after 24 hours
  - This is a shorter period of induction than in other experiments - but text does say no further increase after longer periods (data not shown) - that would imply that concentration of p21 decreases over time if cell volume increases?
  - this analysis compares amount of p21 per cell – but for this experiment could/should have used equal protein loading for this experiment maybe?
- very little p21 in –IPTG cells means that
  - expression tightly regulated - good news – inducible system is working! and

- very little endogenous p21 – as expected? (would not expect high levels in normal, unstressed, proliferating cells)
  - in practical write-up most students completely missed the point that CHO cells have their own endogenous p21 gene (even though it is hamster not mouse it is the same thing, but under normal control!)

Fig 3.

- straightforward – transfection / selection has not resulted in anything odd / unexpected or different

Fig 4.

- -IPTG shows normal growth curve of exponential followed by stationary phase (as nutrients become depleted and toxic metabolites build up (and some cell death kicks in?))
- IPTG/p21 effect is rapid and reversible – ie the system is working

Fig 5.

- note drop in %S as cells enter stationary phase – refer back to reasons for slow down / arrest of cell proliferation in control cultures (fig 4)
- descriptive account of progressive decline in cycling cells +IPTG, as they become trapped in predominantly G1-phase but also G2-phase (both S and M blocked by p21)

Fig 6. FLAW IN INTERPRETATION – IT IS VOLUME NOT DIAMETER THAT IS IMPORTANT ( $\pi r^3$ )

- 1.6X increase in diameter = 3.9X increase in volume at 72 hours
  - i.e. comparable with 4-fold increase in productivity
- text states that increase in average size (1.6x) is equivalent to slightly less than the increase required to support 2 cell doublings
  - 2 doublings in 72 hours = 4X increase in volume not 2X

Figs 7 and 8. FLAWED EXPERIMENTAL DESIGN

- should have used an equivalent time of 72 hours to compare with productivity and volume increases
- 1.6 – 2-fold increases in mitochondria / ribosomes at 48 hours cannot be directly compared increase in productivity and volume at 72 hours (may well be equivalent increases in these parameters)
  - equal cell loading is in this case more appropriate

## 5. Conclusions

- uncoupling of growth and division has been demonstrated
- increased capacity for protein synthesis per cell is the likely reason for increased productivity
- there is likely to be a closer correlation between the increased productivity and cell size than indicated by the relationship reported (using volume rather than diameter as a measure of size)