Context

Model systems are useful tools for biological research. A model system is designed to allow aspects of complex biological processes to be replicated in a simplified form that is therefore easier to control. Examples of model systems can include anything from individual cells designed to mimic specific functions of differentiated tissues through to the use of genetically modified animals to model human diseases.

In this study, research is being carried out to create a model system that can be used to investigate some of the molecular mechanisms that are damaged in type II diabetes. Specifically, the research aims to identify the mechanisms by which insulin release is triggered in the pancreas and how this changes in response to the strength of the stimulus.

Model of pancreatic function

In the body insulin is synthesised and released from the beta cells found in the Islets of Langerhans within the pancreas. The release of insulin is designed to be proportional to the blood glucose levels in the organism and is regulated by the pathway shown in Figure 1.

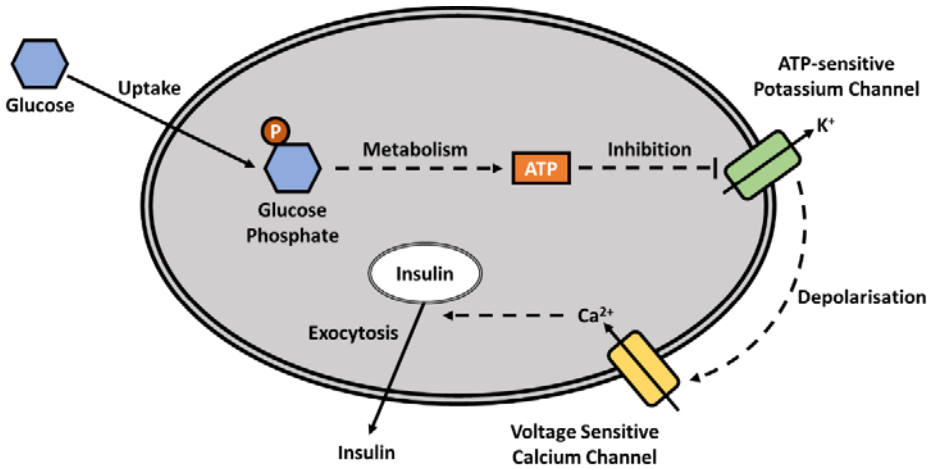

Figure 1: Regulation of insulin release from a beta-pancreatic cell. Glucose is taken into the cell and is metabolised to produce ATP. Increased levels of ATP cause the inhibition (closing) of ATP-sensitive potassium channels and depolarisation of the cell. The depolarisation activates (opens) Voltage Sensitive Calcium Channels allowing calcium ions to enter the cell, triggering exocytosis of insulin from vesicular storage.

The switch from the ATP-sensitive potassium channels being open or closed is very well coordinated in the cell. This suggests that there is a mechanism by which individual potassium channels can interact with one another. To study these interactions a model has been developed based on fibroblasts (skin cells) that have been genetically modified to produce fluorescently labelled ATP-sensitive potassium channels. These function in the same way as potassium channels normally found in beta cells, but also incorporate a protein that can fluoresce when excited with a specific wavelength of light.

Model cell design

Each model cell is modified to produce multiple copies of four different versions of the fluorescent potassium channel. Each of these channels is a different "colour" and therefore has its own excitation and emission spectra based on the structure of the specific fluorophore (see Table 1). It is therefore possible to look at not only the distribution of the proteins in the cell but also identify the locations in which the proteins are found together (colocalised).

Fluorescent Protein	Excitation Wavelength	Emission Wavelength	Brightness
Blue	399	544	32.4
Green	488	507	15.1
Yellow	513	552	50.9
Red	587	610	33.6

Table 1: Excitation and Emission wavelengths (in nm) for the four different fluorescent potassium channels. The brightness of each colour is also given in standard units.

Before carrying out experiments the model cells need to be evaluated to verify they are producing the correct proteins. From a large sample of cells, individuals can be scanned to record the intensity of fluorescence from each of the four labelled potassium channels. This information is used to establish a distribution within the population of cells. The results of this experiment for the four different coloured proteins are shown in the graphs of Figure 2.

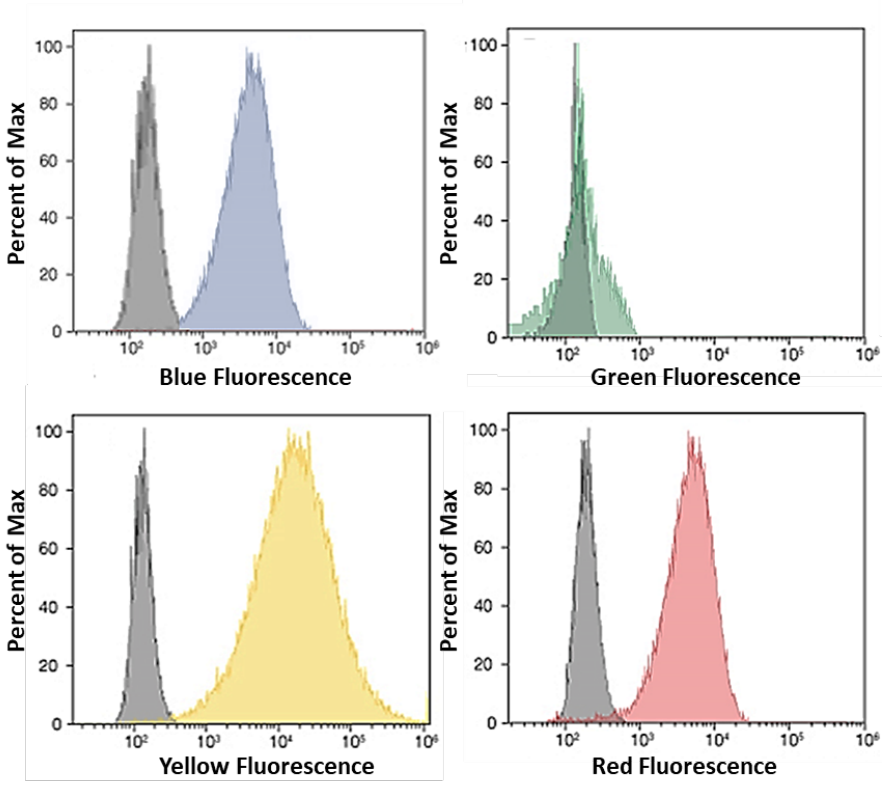

Figure 2: Histograms showing the distribution of individual cells in relation to the fluorescence intensity. Y-axis is scaled so that the largest number of recorded samples for each graph is set to 100%. Each graph includes two sets of data; unmodified cells that lack a fluorescent ATP-sensitive potassium channels (grey) and those with the modified fluorescent version (coloured). Fluorescence intensity (x-axis) is on a logarithmic scale.

Investigating ATP-sensitive potassium channel activation

Once a suitable population of cells has been identified the researchers can use this to investigate the activation of the ATP-sensitive potassium channels. The researchers want to investigate the relationship between the strength of an activating signal and the amount of colocalisation of the ATP-sensitive potassium channels. To answer these questions, they must first establish a method for activating the model cells.

The cells can be activated in two ways:

1) They can be exposed to increased levels of glucose in their growth medium, simulating a rise in blood glucose and activating the pathway described in figure 1.
2) They can be activated by addition of an experimental drug identified in a screen for chemicals that can stimulate insulin release. The exact mechanism by which the drug stimulated insulin release is unknown at the start of the investigation.

Experiment 1: Investigating activation by experimental drug

First the researchers observe the changes to the cells upon addition of the experimental drug. They do this using a confocal microscope that uses a system of lasers to excite cells at specific wavelengths and then records emitted fluorescence. As fluorescence must come from the modified ATP-sensitive potassium channels this information can be used to map their locations within the cell.

An individual cell is located using the microscope and images recorded before the drug is added, and then every 20 seconds after addition. Images from each of the four lasers are overlaid to create a series of composite images as shown in Figure 3.

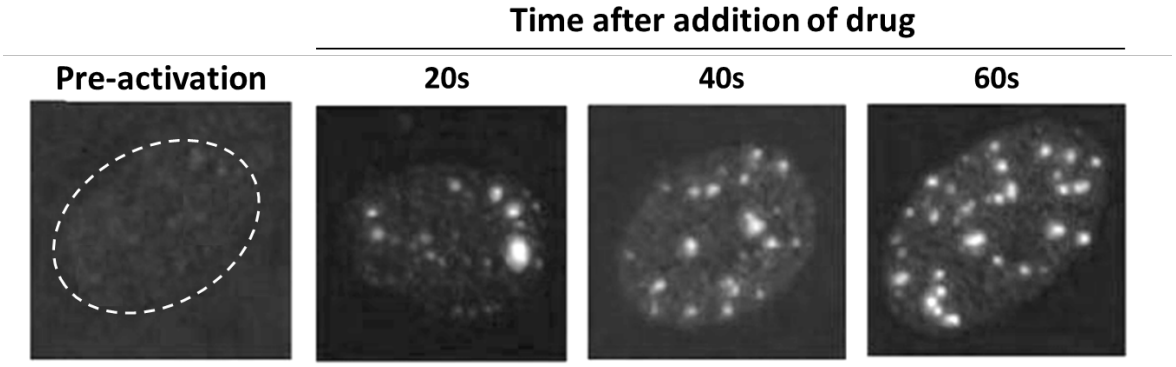

Figure 3: Image showing changes in distribution of fluorescent ATP-sensitive potassium channels within model cell upon addition of experimental drug. Images taken before addition of drug (pre-activation) and every 20 seconds after addition. For the pre-activation image the outline of the cell is shown with a white dotted line. Images represent a composite from four pictures of the same cell, each excited using one of the four wavelengths described in table 1.

Experiment 2: Investigating effect of variation in stimulus

In a second experiment the researchers use an automated method to look for the colocalisation of the ATP-sensitive potassium channels. Samples of the cells that were characterised in Figure 2 are washed in a buffered saline solution and then placed in new growth media that has been supplemented with varying concentrations of glucose or supplemented with the experimental drug. After 5 minutes the cells are cooled on ice, washed again in buffered saline solution and fixed. Fixing is a process where cellular components are chemically crosslinked preserving a snap-shot of the cell.

An automated confocal microscope then scans ten cells at each of the four wavelengths, aligns the pictures and scores areas where there is an overlapping signal from different fluorescent proteins. These data are represented in Figure 4.

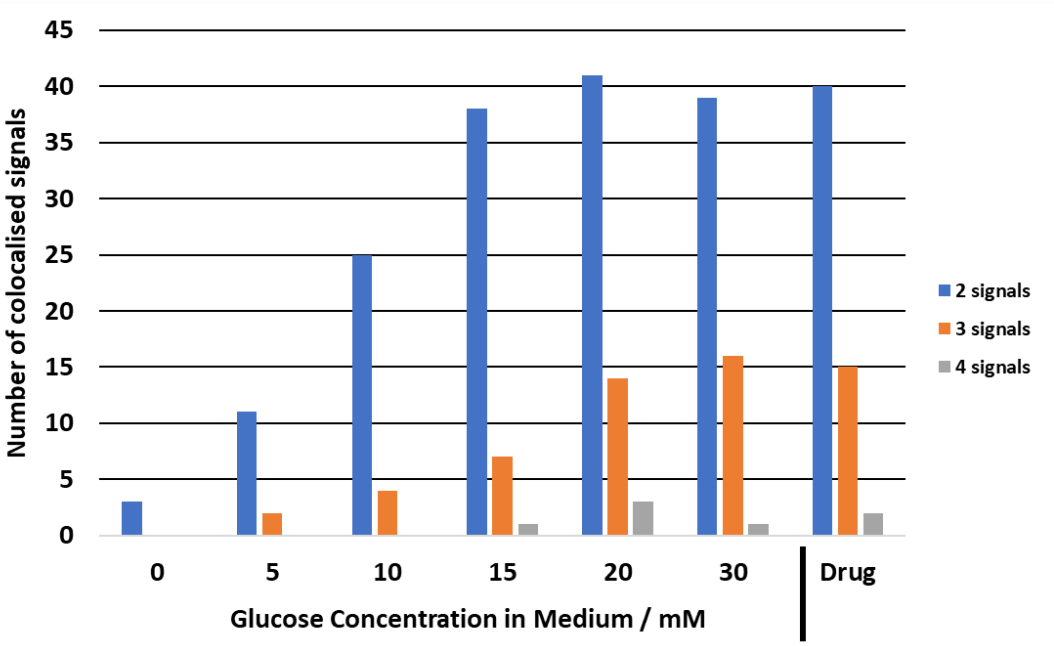

Figure 4: Graph showing the number of colocalised signals in the scanned cells. For each concentration of glucose, 10 cells were fixed and imaged at each of the four wavelengths corresponding to the fluorescently modified ATP-sensitive potassium channels. A colocalisation is recoded if two, three or four signals were detected as overlapping between images. A sample of cells were treated with the experimental drug, shown in the right-hand column.

Next Steps:

From these experiments the researchers believe they have enough information to create a good model system for quantifying the level of activation of insulin from beta pancreatic cells based on different stimuli strength. The next stages will be to investigate the role of mutations in the ATP-sensitive potassium channel and then to transfer this model from a cell based system into a whole animal model.