

Are adult pancreatic beta cells formed by self-duplication or stem cell differentiation?

Introduction

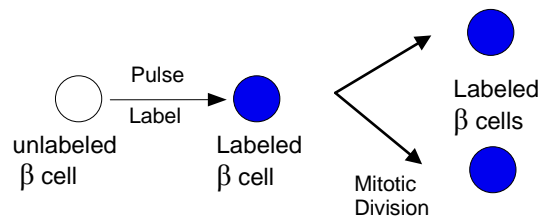
Researchers have long been interested in how tissues produce and maintain the correct number of cells in order to preserve homeostasis. It is known that new cells can be produced by the mitotic division of existing differentiated somatic cells or by the differentiation of stem cells.

There are two broad types of stem cells found in humans and other mammals—embryonic stem cells and adult stem cells. In a developing embryo, stem cells differentiate into all of the specialized tissues of the organism. Once the organism is born, adult stem cells act as a repair system, replenishing specialized cells and also maintaining the normal cell turnover in some organs and tissues such as the blood, skin, and intestinal lining.

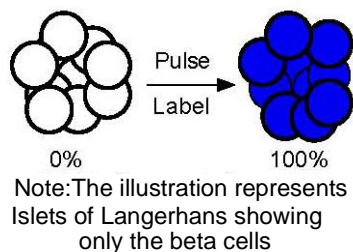
Pulse-Chase Experiment Design

Dr. Melton wanted to determine how insulin-producing beta cells are replenished in the pancreas. He asked: Are there adult pancreatic stem cells that differentiate into insulin-producing β cells? Or are β cells replenished by the mitotic division of other β cells?

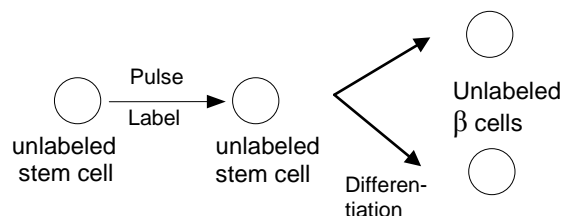
In order to answer this question, Dr. Melton and his colleagues devised a sophisticated pulse-chase experiment. They needed a way to irreversibly pulse-label all existing insulin-producing β cells, so that when they divided, all of the daughter cells in future generations would also be labeled.



Islets of Langerhans consist mostly of insulin-producing β cells. They will appear to be densely labeled after the pulse-label.



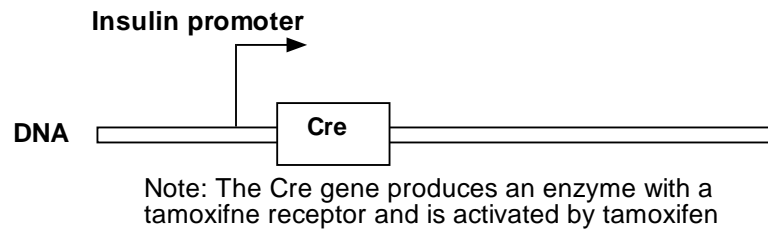
Adult stem cells that can give rise to β cells—if they exist—would not be labeled by this procedure, and thus, any β cells that derive from stem cells after the pulse-label would not be labeled either.



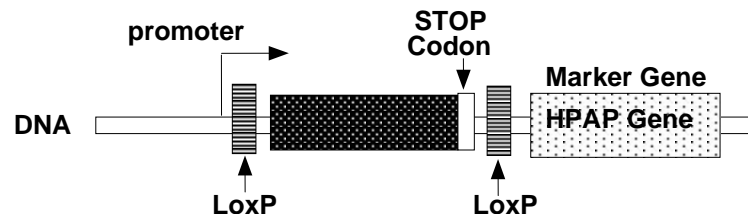
Pulse-Chase Experiment Mechanism

Dr. Melton's team designed a system in which an injection of the chemical tamoxifen (the pulse) irreversibly labeled only β cells. They created a transgenic mouse by inserting two pieces of genetically engineered DNA into its genome.

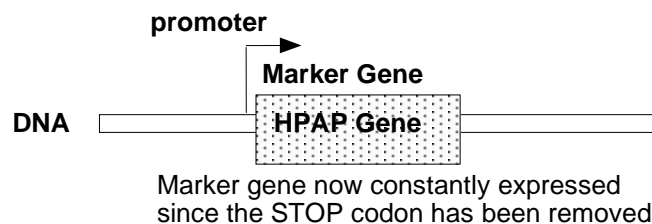
- One piece of DNA carried a gene that produces a version of an enzyme called Cre that requires tamoxifen for activation. Cre recognizes a pair of specific DNA sequences and splices out what's between them.
- They coupled the Cre gene with a copy of the insulin promoter that is normally coupled to the insulin gene. Because the insulin promoter is only active in pancreatic β cells, Cre is only expressed in the pancreatic β cells of the transgenic mice. Cre however is not active until it encounters tamoxifen. (See diagram below.)



- The second piece of DNA carried the marker gene. This gene is coupled with a promoter that is constitutively active. When active, this gene produces a protein called HPAP that can be stained blue. However, the DNA was made so that the marker gene is in tandem with another gene directly upstream from it. The marker gene is not expressed because the other gene's STOP codon prevents the marker gene from being transcribed. The upstream gene is flanked on both sides by special DNA sequences (LoxP) that are recognized by Cre. (See the diagram below.)



- When tamoxifen is injected into the mice, Cre is expressed in β cells. Cre recognizes the special sequence (LoxP) flanking the gene upstream from the marker gene and splices the upstream gene out, including the STOP codon. The marker gene no longer has the STOP codon in front of it. This switches the marker gene on and leaves it on permanently. The β cells can now be stained blue.



- Because Cre is only expressed in insulin producing β cells, the marker gene can only be turned on in β cells.
- Since the activation of the marker gene is due to a permanent change in the DNA, it is passed on to all new β cells produced through the mitotic division of existing β cells.

- Stem cells do not produce insulin. Therefore the marker gene cannot be turned on in stem cells when tamoxifen is applied. When stem cells divide and subsequently differentiate into β cells, tamoxifen is no longer there. It is present only when initially introduced into the mouse during the pulse. They will not be expressing the marker gene and will not be stained blue.

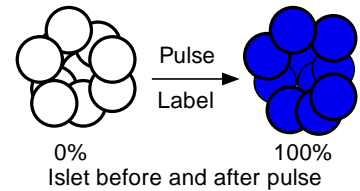
The investigation conducted by Dr. Melton and his colleagues can be thought of as a pulse-chase analysis because they examined a cellular process occurring over time. During the pulse phase of the experiment, they exposed β cells to tamoxifen. This resulted in the production of a chemical that labeled only cells that were producing insulin at that time. The time spent waiting for existing β cells to be replaced by new β cells was the chase.

The Experiment—the Prediction

The diagram below represents an islet in the pancreas. Only β cells are shown. They are represented by small circles. Dr. Melton defines an islet as a group of 10 or more β cells.

The tamoxifen injection causes the expression of the label in insulin-expressing β cells present at the time of the pulse. Any new β cells produced by the mitotic division of these cells will also express the label. The change is a permanent one.

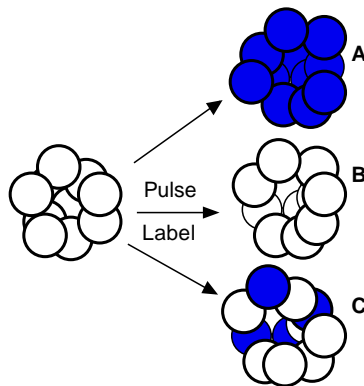
If everything worked perfectly, when the mice were analyzed immediately following the injection of tamoxifen, 100% of the β cells would be labeled.



Depending on the source of new β cells, Dr. Melton predicted that after the passage of different amounts of time, one of three outcomes would be possible:

- New β cells would be derived entirely through the mitotic division of existing β cells which were making insulin at the time of the pulse, and are therefore labeled.
- New β cells would be derived entirely from stem cells, which were not producing insulin at the time of the pulse, and therefore are not labeled.
- Both stem cells and existing β cells would replenish β cells.

Depending on the source of new β cells, Dr. Melton and his colleagues expected to see something similar to either A, B, or C a month after the tamoxifen pulse. A, B, and C each represent one of the three predicted outcomes.



- For each one, indicate if it represents replacement by (1) only the mitotic division of existing beta cells, (2) entirely by the division and differentiation of stem cells or (3) a combination of the two. Support your answer.

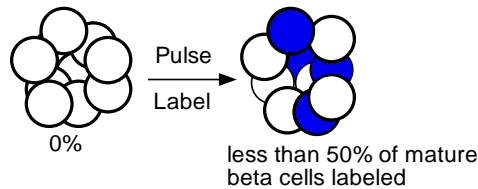
A _____

B

C

The Experiment—Actual Results

When the mice were analyzed immediately following the injection of tamoxifen, it was discovered that every islet contained labeled cells. Dr. Melton and his colleagues were disappointed that an average of less than 50% of the mature beta cells in each islet were labeled. From these results, they concluded that the labeling procedure was less than 50% efficient.



There are a number of reasons why 100% of the mature beta cells were not labeled. One possible explanation is that the dosage of tamoxifen used was not concentrated enough to reach all of the beta cells. Perhaps a higher concentration would be toxic to the mice. Another possibility might be that the STOP codon was excised in less than 50% of the cells. Thus approximately 50% of the cells would be labeled. The exact number of labeled beta cells varied from mouse to mouse. What Dr. Melton had to look for at the conclusion of the chase was not quite as obvious as it would have been if 100% of the mature beta cells were labeled with the blue stain.

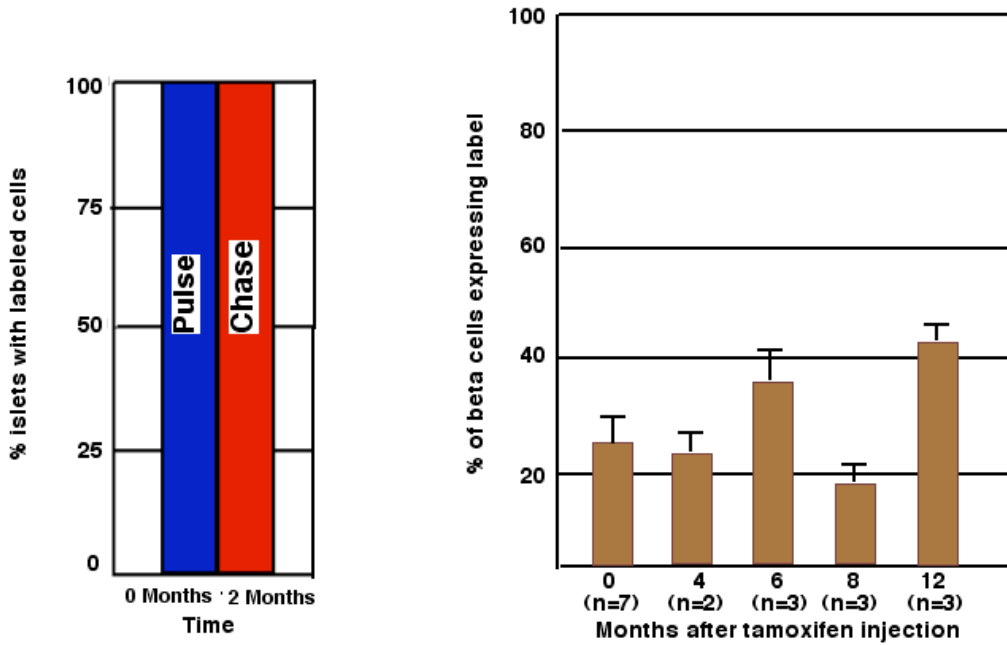
2. Explain what would be expected to happen to the percentage of labeled beta cells in the islets if new beta cells are the result of the mitotic division of existing beta cells.

3. How would the results be different if the source of new beta cells involved both the mitotic division of existing beta cells and stem cells?

4. Explain what would happen over time if all new beta cells resulted from differentiation of stem cells.

Dr. Melton and his colleagues analyzed the mice at 0, 4, 6, 9, and 12 months after tamoxifen injection. The graphs below represent what they found.

Average percentage of islets and beta cells per mouse expressing the label



5. Which type of beta cell replacement is supported by the data? Explain.

6. Using evidence from Dr. Melton’s pulse-chase analysis to support your answer, explain which cell type—adult stem cells or differentiated β cells—should be chosen to investigate as a possibility for cell-based therapy for type I diabetes.

7. (a) Identify a problem associated with the use of differentiated β cells for possible cell-based therapies for type I diabetes.

(b) Identify a problem associated with using adult stem cells for possible cell-based therapies for type I diabetes.
