

The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles by J. B. Gurdon (1962)

In 1962 researcher John Bertrand Gurdon at the University of Oxford in Oxford, England conducted a series of experiments on the developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. In the experiments, Gurdon conducted nuclear transplantation, or cloning, of differentiated cells, or cells that have already specialized to become one cell type or another, in tadpoles. Gurdon's experiment showed that differentiated adult cells could be induced to an undifferentiated state, where they could once again become multiple cell types. Gurdon's experiment disproved the theory that differentiated cells could not be undifferentiated or dedifferentiated into a new type of differentiated cell. Gurdon's experiment demonstrated nuclear transplantation, also called cloning, using differentiated cells.

In 1960, Gurdon obtained his doctorate in zoology at the University of Oxford after researching new techniques of nuclear transplantation in *Xenopus laevis*, a species of frog. Gurdon conducted his experiment with tadpoles in the embryology laboratory at the Department of Zoology at the University of Oxford.

Gurdon's experiments built on research conducted by Robert Briggs and Thomas King who performed the first nuclear transfer in living organisms in 1952. Nuclear transfer is the process of transplanting the nucleus of one cell into an unfertilized enucleated egg cell, a cell whose nucleus was removed. Prior to Gurdon's experiments, Briggs and King had argued that nuclear transfer was impossible if the cells used in the transplantation had already developed beyond a certain point. The theory, supported by Briggs and King's experiment, was that once a cell differentiates, the cell could no longer differentiate again. Gurdon questioned whether cells lose certain genes after they specialize that consequently prevent them from transforming into new cell types.

Gurdon's series of experiments aimed at determining whether cells, as they develop and specialize, lose the ability to produce different cell types. In his experiments, Gurdon transplanted nuclei from tadpole cells of *Xenopus*, and other frog species, into unfertilized *Xenopus* eggs and observed how the modified eggs proceeded to develop. Gurdon stopped the development of some the modified eggs, or fixated them, and allowed others to develop as far as they were able. He compared them and noted trends in development based on the conditions of the fixed eggs. Gurdon also tested whether using nuclei of eggs produced through nuclear transfer to produce successive generations of eggs led to eggs that developed to a further stage.

Gurdon collected donor cells from the mid-intestine of *Xenopus* tadpoles, a developmental stage of frogs preceding adulthood. Gurdon selected mid-intestine cells because they were larger and easier to see due to a specific striation pattern that differed from other cell types present in the intestine. Gurdon noted that the quality of *Xenopus* eggs laid in the laboratory were of varying quality due to the artificial conditions of the laboratory. To account for that variability, Gurdon also used cells from the same developmental stage that Briggs and King had used to serve as the control group.

In the first experiment, Gurdon performed nuclear transfer using a donor mid-intestinal nucleus from a *Xenopus* tadpole and an unfertilized egg of the same frog species. To accomplish nuclear transfer, Gurdon first softened the outer layer of the unfertilized egg with UV radiation. That step degraded the nucleus of the egg and also weakened the cell membrane, enabling Gurdon to inject a nucleus from a different cell into the unfertilized egg cell using a pipette, or a small needle-like

measurer. After transplanting nuclei into many egg cells, Gurdon stopped the development of some of the modified eggs, a process called fixation. By fixating the eggs, Gurdon was able to study the modified egg at an exact point in time. Gurdon allowed the remaining eggs to develop normally and then compared the fixed and unfixed eggs. Gurdon sliced the fixed eggs into sections and viewed the sections under a microscope. He found that many of the transplanted nuclei had abnormalities. Gurdon concluded that certain abnormalities in the transplanted nuclei led to specific abnormalities in development.

Gurdon noted several trends when he compared the fixed eggs to the unfixed eggs. He found that many of the eggs did not experience cleavage, the process of egg division, after transplantation. Cleavage enables a fertilized egg to divide and produce the many cells that will make up the organism. Gurdon concluded that the lack of cleavage was the result of a technical fault in which the transplanted nuclei were not effectively exposed to the cytoplasm, or cellular material within the cell, of the egg. The cytoplasm of the egg is crucial in the process of cleavage because it signals the nuclei to induce cleavage. Gurdon also noticed that some eggs did not have a nucleus and he concluded that the absence of the nucleus was most likely due to the accidental removal of the nuclei after transplantation due to the nucleus sticking to the pipette. Those technical errors caused eggs not to develop beyond a certain stage, halting their development.

In the second experiment, Gurdon transplanted the intestinal nucleus from a different species of frog, called *Hymenochirus curtipes* into the unfertilized *Xenopus* eggs to see whether using different species affected the rates of abnormal cleavage. By using nuclei from the frogs of another species, Gurdon could observe the genetic differences between species led to abnormal cleavage. Gurdon transplanted the nuclei of *H. curtipes*, and the nuclei of *Xenopus* into unfertilized *Xenopus* eggs. *Xenopus* eggs that received *H. curtipes* nuclei experienced early arrest, while many of the *Xenopus* eggs that received *Xenopus* nuclei developed normally. Gurdon noted that the percentage of transfers that experienced halted development was the same in each nuclear transfer. Once the eggs reached the blastula stage, or the early embryonic stage during which a hollow sphere of cells form, the eggs that received the genetically different donor nuclei from the different frog species stopped developing. The eggs receiving the genetically identical donor nuclei from the same species continued to develop. He concluded that genetically different donor nuclei did not have higher rates of abnormal cleavage than genetically identical donor nuclei, which meant that the genetic difference did not cause the abnormal cleavage.

Gurdon performed the third experiment to determine whether the quality of eggs increased or decreased with each successive generation. To do so, he enucleated eggs as he had done previously and inserted donor nuclei into the eggs. Gurdon then allowed the modified eggs to develop. He referred to those as first-transfer eggs. He then removed the nuclei of the first-transfer eggs and performed nuclear transfer once again, a step he called serial-transfer. That gave rise to the first serial-transfer generation of eggs. Gurdon found that in all cases, the serial-transfer eggs developed to a further stage than the first transfer eggs.

Gurdon posed two possible explanations for the results of the third experiment. The first was that the nuclei's ability to develop increased after nuclear transfer was performed, causing the serial-transfer eggs to develop further than the first-transfer eggs. The second possibility was that the abnormality of the first-transfer embryo was due to poor egg quality or to genetic causes.

To determine which explanation was correct, Gurdon created multiple serial-transfer generations of eggs. The later generations did not contain more abnormal cleavage events than did the earlier generations. From those results, Gurdon concluded that developmental capacity does not increase as a result of multiple, serial-transplants.

Based on the results of the three experiments, Gurdon concluded that the cell types produced after transplantation indicated the genetic information contained within the transplanted nucleus. Likewise, he argued that the transplanted nucleus must contain the information supporting the development of a normal tadpole.

Gurdon hypothesized that some cells may become differentiated under the influence of neighboring cells due to cell to cell communication, which would explain why some cells in a specific tissue contain the nuclei that have the genetic information to form a normal tadpole. Further, Gurdon argues

that the cytoplasmic environment of a cell initiates differentiation and that the nucleus provides the information to code for a particular cell type.

The experiment showed that cloning could be performed with the nucleus of more types of cells than previously thought. In 1997, the technology used by Gurdon in his 1962 article was later used in the cloning of a sheep named Dolly, demonstrating the wide range of possibilities made possible by nuclear transfer.

Sources

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