

# Shoukhrat Mitalipov and Masahito Tachibana's Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells (2009)

In 2009, at Oregon National Primate Research Center in Beaverton, Oregon, Shoukhrat Mitalipov, Masahito Tachibana, and their team of researchers replaced the mitochondrial genes of primate embryonic stem cells via spindle transfer. Spindle replacement, also called spindle transfer, is the process of removing the genetic material found in the nucleus of one egg cell, or oocyte, and placing it in another egg that had its nucleus removed. Mitochondria are organelles found in all cells and contain some of the cell's genetic material. Mutations in the mitochondrial DNA can lead to neurodegenerative and muscle diseases. Mitalipov and Tachibana used spindle replacement to produce healthy offspring from an egg with mutated mitochondria in rhesus macaques (*Macaca mulatta*). The experiment showed that spindle transfer eliminated the chance of transmission of mitochondrial diseases from the affected primates to their offspring, offering the potential to eliminate mitochondrial diseases in humans.

The experiment focused on mitochondria, which are energy-producing organelles found in all eukaryotic cells that are important to normal development. The mitochondria contain thirty-seven genes responsible for energy production in cells. Occasionally, the genetic material found in the mitochondria contains mutations. Mitochondria are maternally inherited, meaning they are passed from mother to offspring. Mitochondria contain approximately .01 percent of a cell's genetic material, which is called mitochondrial DNA. Each mitochondrion contains between two and ten copies of mitochondrial DNA. Each cell, containing many mitochondria, can contain thousands of copies of mitochondrial DNA. Because of the large number of copies of mitochondrial DNA, mutations occur in the mitochondrial DNA ten times more often than in the nuclear genome, the DNA of the cell's nucleus. Researchers associate many mutations of mitochondrial DNA with specific disorders, including myopathies, or muscular disorders, neurodegenerative diseases, and diabetes.

To identify mitochondrial DNA mutations within a cell, researchers analyze the genetic material of mitochondria from that cell. A cell normally contains many copies of the same mitochondrial DNA, called homoplasmy. When a cell contains two or more types of mitochondrial DNA, called heteroplasmy, researchers are alerted that mutated mitochondria are present. When enough cells contain mitochondrial mutations, different tissues can be affected, which can result in mitochondrial diseases.

In 2009, Mitalipov and Tachibana studied rhesus macaque eggs with mitochondrial DNA mutations. The researchers used the technique of spindle transfer to successfully transfer the nuclear DNA in the affected egg into a donor egg containing healthy mitochondrial DNA. Mitalipov and Tachibana hypothesized that nuclear DNA from an egg with mutated mitochondrial DNA could be transplanted into an egg with healthy mitochondrial DNA through the use of spindle transfer technique, which would eliminate the possibility of passing the mutated mitochondrial DNA from mother to offspring. When using spindle transfer, researchers first remove the genetic material from an egg cell that contains heteroplasmic, or a mix of healthy and mutated mitochondrial DNA. Then the researchers transplant that egg cell's genetic material into a donor egg cell whose nucleus has been removed and whose mitochondrial DNA is undamaged. Following the transfer of genetic information to the egg and after a sperm has fertilized the egg cell, the resulting embryo will develop with healthy mitochondria.

To begin, Mitalipov and Tachibana investigated the distribution of mitochondria around the cell's

cytoplasm within mature rhesus monkey eggs to prevent transferring over mutated mitochondria. They found that mitochondria were evenly distributed across the cytoplasm. They also noted that chromosomes, which contain nuclear DNA, and spindle fibers that separate the chromosomes, lacked mitochondria in the cytoplasm that surrounds them. Those observations led Tachibana and Mitalipov to predict that they could transfer the spindle and chromosomes of a cell without also transferring the cytoplasm containing the damaged mitochondrial DNA. That would enable them to avoid transferring mutated mitochondria into the donor egg. To test their prediction they removed the spindle-chromosomal complex surrounded by a small amount of cytoplasm, a complex called the karyoplast. Tachibana and Mitalipov calculated that only 1.5 percent of the cell's cytoplasm was included in the karyoplast samples, a percentage they determined to be negligible. The researchers concluded that the small amount of mutated mitochondria contained in the 1.5 percent was not enough to affect the outcome of the egg cell.

Next, Mitalipov and Tachibana investigated the process of transplanting the karyoplast into donor cells with their nucleus removed. The team inserted the karyoplast into the egg cell. After placement of the karyoplast in the egg cell, the investigators attempted to facilitate the fusion of the karyoplast to the cell's cytoplasm. To do that, they applied electrical stimulation to the egg cell to encourage permeability, the ability of objects to pass through the cell's membrane. After the fusion of the karyoplast with the donor cytoplasm, Mitalipov and Tachibana observed a problem when the egg cell began meiosis, the process of cell division of reproductive cells that should only occur after the egg is fertilized. The researchers observed premature meiosis. They found that the unintended division of the egg cell produced a polar body, which is a cell that cannot develop into a mature egg.

Mitalipov and Tachibana hypothesized that the electrical stimulation they used to fuse the karyoplast with the donor cell caused premature meiosis, and that they could avoid premature meiosis by using SeV-assisted fusion. SeV-assisted fusion is an alternative method of karyoplast fusion that involves the use of an extract from the Sendai virus, or SeV. The researchers briefly exposed the karyoplast to the viral extract. They found that the viral extract enabled rapid fusion of the karyoplast and the surrounding cytoplasm of the donor egg. The resulting egg successfully remained in its mature state and did not undergo premature meiosis. The researchers concluded that SeV-assisted fusion prevented the premature division of the egg and was therefore a valid means of karyoplast fusion.

Mitalipov and Tachibana then compared the development competence, or the ability of the egg to develop further, of the two processes of karyoplast fusion. They fertilized eggs that underwent electroporation-assisted fusion and eggs that underwent SeV-assisted fusion. The fertilized electroporation-assisted eggs failed to create a pronucleus, the combined nucleus of the sperm and egg cells. Without a pronucleus, the egg would not form an embryo. The fertilized SeV-assisted eggs underwent successful fertilization and development. Those results confirmed that SeV-assisted fusion did not compromise fertilization and embryonic development. That outcome showed that the technology was successful in producing healthy, viable blastocysts. A blastocyst is a clump of cells in the early stage of development in mammals. They contain an outer ring of cells and an inner cell mass.

To assess the quality of the blastocysts produced by SeV-assisted fusion and fertilization, Mitalipov and Tachibana compared the spindle transfer blastocysts to a control group of unaltered blastocysts. The researchers found that the spindle transfer blastocysts contained similar numbers of inner cell mass cells and total number of cells compared to the unaltered blastocyst control group. The comparison showed that the blastocysts developed using their technology were as healthy as blastocysts that did not undergo mitochondrial transfer.

To further examine the development potential, Mitalipov and Tachibana isolated embryonic stem cell lines, or cells with the potential to develop into many cell types. The team isolated them from the spindle transfer blastocysts, as well as from the control group to ensure that the stem cell lines developed into healthy cell lines containing many cell types. Both cell lines exhibited similar levels of pluripotency, or the ability for one type of cell to become many different cell types. The ability of embryonic cells to develop into many cell types is crucial in the development of an embryo.

The researchers then checked to see whether spindle transfer caused chromosome abnormality. Chromosome abnormality occurs when a cell has an abnormal number of chromosomes or when chromosomes are malformed. Observing chromosome abnormality would indicate that the technique damaged chromosomes. The analysis demonstrated that the spindle transfer chromosomes had no abnormalities when compared to the control group.

Next, Mitalipov and Tachibana tested the developmental potential of the spindle transfer blastocysts to see whether they would result in embryos that could be implanted in a female rhesus macaque. They fertilized the spindle transfer blastocysts and transplanted the resulting embryos into the reproductive tracts of two recipient female macaques. On 24 April 2009, the first female macaque, which Tachibana and Mitalipov had implanted with the fertilized egg, gave birth to a set of healthy twin macaques. According to Tachibana, the twins were the first spindle transfer animals to be born with healthy mitochondrial DNA. On 8 May 2009, the second pregnant macaque gave birth to a healthy infant. The three infants were all of healthy birth weight and size. Mitalipov and Tachibana concluded that karyoplasts could be successfully isolated and transplanted into healthy, enucleated oocytes.

Finally, the team sought to determine if any mutated mitochondrial DNA had managed to form in the mitochondrial DNA of the resulting offspring. Mitalipov, Tachibana, and their team found that the nuclear DNA of the offspring matched the genome of the spindle-donor animals. Further, they confirmed that damaged mitochondrial DNA was not present in the mitochondria of the offspring.

Overall, the results of the experiment showed that scientists could replace mutated mitochondrial DNA in mature eggs using the spindle transfer technique. Mitalipov states that the technology has the potential to eliminate the chances of a woman affected by a mitochondrial disease passing the mutation on to her offspring. Although the experiment documented the first case of spindle transfer in rhesus macaques, Mitalipov argues that one day the technology could be applied to humans. The technology can serve as a new therapeutic approach to eliminating the mitochondrial disease before the offspring are born.

## Sources

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