Sonja Vernes, et al.'s Experiments On the Gene Networks Affected by the Foxp2 Protein (2011)

In 2011, Sonja Vernes and Simon Fisher performed a series of experiments to determine which developmental processes are controlled by the mouse protein Foxp2. Previous research showed that altering the Foxp2 protein changed how neurons grew, so Vernes and Fisher hypothesized that Foxp2 would affect gene networks involved in the development of neurons, or nerve cells. Their results confirmed that Foxp2 affected the development of gene networks involved in the growth of neurons, as well as networks that are involved in cell specialization and cell communication. The researchers determined that Foxp2 is important for a variety of developmental processes such as motor control, language acquisition, and cognition.

Foxp2 is a transcription factor protein, which dictate how often certain genes are transcribed into proteins (expressed) in a cell. By controlling the creation of proteins, transcription factor proteins can influence gene networks that in turn influence some biological function. Researchers can determine the function of a transcription factor by identifying the gene networks it controls. The Foxp2 protein is a forkhead box protein, which means that it attaches to genes with three protrusions like the tines of a fork. The Foxp2 protein is made from the Foxp2 gene. Most vertebrate species carry some version of the Foxp2 gene, and the name changes slightly for each. The Foxp2 gene is the mouse version, and the protein that the Foxp2 gene makes is called Foxp2. For humans, the gene is written as FOXP2, and the corresponding protein is written as FOXP2. For other animals, the gene is written as FoxP2, and the protein is written as FoxP2. The gene and protein are nearly the same in all mammals, but there are a few changes in the DNA sequence of the gene between species.

At the time of the experiments, Fisher was the director of the Language and Genetics Department at the Max Planck Institute for Psycholinguistics in Nijmegen, the Netherlands. He and other researchers originally discovered the gene that codes for the human version of the Foxp2 protein, called the FOXP2 gene, in 2001. In 2002, Fisher established his own lab at the Wellcome Trust Center for Human Genetics in London, England, where Vernes joined him to complete her doctoral work. By 2011, Vernes led her own lab at the Language and Genetics Department at the Max Planck Institute for Psycholinguistics. Many collaborators from the principal researchers' respective labs and institutions assisted them throughout the experiments.

Two previous experiments had established that Foxp2 controls genes that are involved in the development of neurons. Researchers led by Svante Pääbo and Wolfgang Enard performed the first experiment in 2002 at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. They engineered mice to carry the human version of the gene that codes for the FOXP2 protein, and their experiment showed that those mice had longer neural outgrowths than mice with the normal mouse protein Foxp2. Neural outgrowths extend from the main body of neurons and send signals to other neurons, communicating messages throughout the nervous system. The experiment's results indicated that changes to the Foxp2 protein affected neural development.

Fisher and colleague David Geschwind performed an experiment in 2009 that showed that the human FOXP2 protein controlled the expression of a gene called CNTNAP2 in human fetal tissues. CNTNAP2 is involved in signaling between neurons in the brain. Neurons communicate with each other using neurotransmitters, a kind of chemical signal. Researchers used brain-imaging studies to determine which neurons interact with each other, and how often. Fisher and Geschwind found that when the human FOXP2 proteins were in the cells of developing mouse embryos, neurons in the mouse brains communicated less often as adults. Fisher and Geschwind's discovery that the

FOXP2 protein controlled the CNTNAP2 gene implied that the human FOXP2 protein controlled genes that themselves cause neurons to develop.

Because both Pääbo and Enard and Fisher and Geschwind's experiments showed that the Foxp2 protein affected neural growth in mice, Vernes and Fisher hypothesized that the Foxp2 protein would affect gene networks involved in the development of neurons. Gene networks are sets of interacting genes within and between cells that control each other's expression and together cause the cells to differentiate into specific types of cells, or to perform specific behaviors. The researchers expected Foxp2 to affect groups of genes that controlled different parts of neural development.

In order to identify which gene networks the Foxp2 protein affect, the researchers identified all of the genes that the Foxp2 protein affects and looked for groups of genes that had similar functions. That process required two steps. First, the researchers had to determine which genes the Foxp2 protein directly influenced, and then which genes the Foxp2 protein indirectly influenced. Analyzing both directly and indirectly affected genes would reveal the full scope of the Foxp2 protein's influence.

To determine which genes the Foxp2 protein directly influenced, the researchers had to identify the genes to which the Foxp2 protein directly binds. Transcription factors proteins control the expression of genes by attaching to the gene's promoter, which is a region that sits in front of the gene on the DNA strand. When the transcription factor attaches to the promoter, enzymes that start transcription are attracted to the promoter, and the gene can be transcribed into a protein. Because each promoter regulates only one gene, researchers can identify which genes a transcription factor protein controls by identifying which promoters the transcription factor protein binds to.

Vernes and her colleagues used a process called chromatin immunoprecipitation (ChIP) to isolate all the promoters that the Foxp2 protein bound to in cells from embryonic mouse brains. The researchers used embryonic mouse brains because they were studying which genes the Foxp2 protein controls during development, and Foxp2 is present in abundance in developing mouse brains. Half of the mice were bred to have the Foxp2 gene to produce Foxp2 protein, while half of them were not. The mice without Foxp2 genes were the control group. The researchers performed all of the following experiments on the tissue samples with the Foxp2 gene, and on the control group. Doing that allowed the researchers to ensure any results of their experiment were actually controlled by the Foxp2 gene and not by another protein.

The researchers harvested the mouse brains on the sixteenth day of development, when Foxp2 protein levels were especially high. They prepared thin slices of brain tissue to use in their ChIP analysis. The researchers broke apart the cells in the tissue using chemicals in order to expose the cells' DNA and transcription factors. They also exposed the cells to a chemical called formaldehyde that linked the Foxp2 transcription factor protein to the places where it was bound on the DNA. The researchers concluded that any place the Foxp2 protein was bound was a promoter for genes that the Foxp2 transcription factor protein controlled directly. After locating those promoters, the researchers used a DNA microarray to identify the DNA sequence of the promoters and determine which genes they controlled. DNA microarrays enable researchers to use already described pieces of DNA, which are called probes, to identify undescribed pieces of DNA. The researchers determined that the Foxp2 protein directly bound to the promoters of 264 genes. They concluded that the Foxp2 protein controlled those genes directly.

The second step was to identify the genes that Foxp2 indirectly controlled. Transcription factors can control the expression of genes that they do not directly bind to, as the products of the bound genes (direct targets) can go on to affect the proteins produced by other genes (indirect targets). The genes that the direct target alters are called indirect targets. Again, Vernes and her colleagues prepared samples of brain tissues from mice that did have Foxp2 proteins and from mice that did not carry any Foxp2 proteins. They created expression profiles for the tissue with the Foxp2 protein and for the control group that did not contain any Foxp2 protein. Expression profiles indicate how much of a protein is being produced in a cell. The researchers compared the expression profiles for the tissue with the Foxp2 protein and the control group and looked for proteins whose production increased or decreased when the Foxp2 protein was absent. The researchers concluded that the Foxp2 protein controlled the genes of all those proteins, either directly or indirectly. Then, they

removed the genes they knew the Foxp2 proteins directly bound to, based on the first part of their experiment. The remaining 321 genes were all indirect targets, because the Foxp2 protein affected their expression but did not directly bind to their promoters.

Once the researchers identified the direct and indirect targets of the Foxp2 protein, they determined the functions of those genes. Both sets of genes were involved primarily in four processes. The first was cell migration, the movement of cells from one location to another. Cells migrate as tissues develop in embryos, and later to heal wounds and for immune responses. The second process was a cell's ability to use receptors on its surface to communicate with other cells. The third process was axon guidance. Axons are the part of the neuron that send signals to other neurons, and axon guidance is the way that axons are positioned within neural networks. Lastly, the Foxp2 target genes were involved in the development of neurons and neurites, small outgrowths that eventually become axons or dendrites, the parts of neurons that receive signals from other neurons. Most of the genes from each data set affected neurons, so Vernes and Fisher proposed that neural development was the largest biological process affected by the Foxp2 protein.

In 2011, Vernes and Fisher published an article that described their results, ”Foxp2 Regulates Gene Networks Implicated in Neurite Outgrowth in the Developing Brain.” As of 2015, the research influenced research on a variety of topics, including brain development, the evolution of language, and autism spectrum disorder. Autism spectrum disorder is a neurodevelopmental disorder that inhibits a child from communicating and interacting with others. In 2012, scientists found that some of the genes affected by the Foxp2 protein are damaged in individuals with autism spectrum disorder.

Sources

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