Equilibrium Density Gradient Centrifugation in Cesium Chloride Solutions Developed by Matthew Meselson and Franklin Stahl

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Matthew Meselson, Franklin Stahl, and Jerome Vinograd, developed cesium chloride, or CsCl, density gradient centrifugation in the 1950s at the California Institute of Technology, or Caltech, in Pasadena, California. Density gradient centrifugation enables scientists to separate substances based on size, shape, and density. Meselson and Stahl invented a specific type of density gradient centrifugation, called isopycnic centrifugation that used a solution of cesium chloride to separate DNA molecules based on density alone. When Meselson and Stahl developed the technique in the mid-1950s, scientists had no other way to separate macromolecules that were of similar size but varied in density. Meselson and Stahl employed their method to determine how DNA replicates, became known as the Meselson-Stahl experiment. Density gradient centrifugation using cesium salts allowed scientists to isolate DNA and other macromolecules by density alone.

Density gradient centrifugation requires the use of a centrifuge, an instrument that spins mixtures in a rotor to concentrate or separate materials. The spinning causes sample solutions in tube or bottle shaped containers to experience a centrifugal force that pushes samples away from the center of the rotor toward the bottom of the tube. Centrifugal force causes components of a mixture to separate by size because larger components experience greater centrifugal force than smaller components. Thus, centrifugal force pushes the larger components of a mixture farther from the rotor and closer to the bottom of the tube.
While many laboratory procedures use conventional centrifuges, density gradient centrifugation requires a special type of centrifuge called an analytical ultracentrifuge or ultracentrifuge. Theodor Svedberg at the Uppsala University in Uppsala, Sweden invented analytical centrifuges in the mid-1920s, which contributed to him winning the Nobel Prize in Chemistry in 1926. Analytical ultracentrifuges can spin samples around five times faster than standard centrifuges. The faster a centrifuge spins, the more force exerted on the sample. The increased force not only provides clearer separation by condensing components of a mixture, and causes solutions to form density gradients during centrifugation. The definition of density is the amount of matter per unit volume of a substance. Dense substances have more matter in a given space than less dense substances. During centrifugation, a density gradient forms in solution when the density of that solution gradually increases moving farther from the centrifuge rotor. In addition to forming density gradients and separating substances, many analytical ultracentrifuges enable scientists to analyze their samples during the process of centrifugation. When performing density gradient centrifugation, researchers use the ability to observe their samples during centrifugation and to watch the order that the components of their samples separate over time.

Before scientists invented density gradient centrifugation in the 1950s, scientists used differential centrifugation to separate substances in a mixture to identify, characterize, and analyze the mixture’s components. Differential centrifugation separates particles of different sizes or shape and isolates them from the liquid in a mixture. Differential centrifugation works well if the particles are greatly different in size. However, scientists could not use differential centrifugation to separate macromolecules of similar sizes or shape, such as DNA molecules.

Several researchers began developing methods to overcome the size and shape problem. Myron Brakke, a protein scientist at the Brooklyn Botanic Garden in Brooklyn, New York, invented the first kind of density gradient centrifugation in 1951. Brakke’s method, called rate zonal centrifugation, separated particles based on size and shape in a relatively dense solution along a density gradient. The solution formed a density gradient for two reasons. First, the centrifugal force pushing down on the solution increased the pressure moving farther from the rotor, it thereby condensed more of the solution in a smaller amount of space. Second, the solution closer to the rotor compressed the solution farther from the rotor. In a dense solution, particles move slow enough for their varying speeds to be measurable. Rate zonal centrifugation used the different speeds at which particles moved away from the rotor, determined by size and shape, to separate components of a solution. However, it could not separate macromolecules based on density alone, because density does not necessarily depend on the size and shape of a particle.

Scientists started to understand DNA structure and knew that genes were made of DNA when Meselson and Stahl conducted their DNA research. However, scientists did not understand how DNA copied itself to pass on the inherited traits stored in the DNA. Meselson and Stahl created their density gradient centrifugation method to better study DNA. In 1953, James Watson and Francis Crick, two scientists at the University of Cambridge in Cambridge, England, proposed a mechanism by which DNA replicated itself. That self-replication of DNA explained how the molecule could pass on its genetic information. Throughout the mid-1950s, scientists proposed different mechanisms to explain the same self-replication phenomenon. Meselson and Stahl used density gradient centrifugation to analyze DNA during replication and test the different replication mechanisms.
The density gradient centrifugation method that Meselson and Stahl developed separated DNA based on density alone. Meselson and Stahl, two postdoctoral fellows at Caltech, hypothesized that they could tag DNA as it replicated and then trace the tag over many replication cycles to see what role the original DNA molecules played in replication. The researchers suggested tagging the original DNA with heavy elements that would not significantly alter the DNA’s chemical properties. While heavy elements would increase the weight of the DNA, they would not increase the size of the molecule, thereby only increasing the density of the original DNA. Meselson and Stahl planned to stop tagging DNA in subsequent replication cycles, which meant that once DNA replicated, the samples would contain some of the original heavy DNA and some light DNA. Therefore, Meselson and Stahl needed to develop a new separation technique capable of separating DNA based on density rather than size or shape.

In developing their new density gradient centrifugation technique, Meselson and Stahl first chose a dense solution where the DNA molecules would separate. The solution needed to be dense enough for the DNA molecules to float before centrifugation. Because Meselson and Stahl studied DNA from viruses and bacteria, the solution also needed to be mild enough so the microbes could survive. In 1956, Meselson and Stahl chose a cesium salt solution. When salt dissolves in a liquid the volume changes slightly but the mass increases resulting in a greater density solution. Because cesium is a heavy element, a cesium salt solution is much denser than the density of most salt solutions and the cesium salt solution did not affect viruses or DNA. Cesium salt solutions had ideal properties for studying DNA.

After picking their dense solution, Meselson and Stahl needed to determine which centrifuge to use for their technique. The researchers used centrifuges manufactured by the Specialized Instrument Corporation, or Spinco, a company founded by Edward Greydon Pickles and Maurice Hanafin in 1946, that became part of Beckman Instruments in 1954. Meselson and Stahl chose the Spinco Model E analytical ultracentrifuge, a model of ultracentrifuge built in 1956. At six feet high and over seven feet long, the Spinco Model E analytical ultracentrifuge spun samples up to 60,000 rotations per minute, which was on the higher end for ultracentrifuges at the time. In addition, the Spinco Model E ultracentrifuge obtained those speeds with very little error and contained a temperature and pressure controlled chamber for the samples. Those qualities ensured that the DNA separated at the right rate and that it retained its structure due to a consistent temperature. For learning how to operate the ultracentrifuge, Meselson and Stahl consulted Jerome Vinograd, the ultracentrifuge expert at Caltech.

When spun rapidly in an ultracentrifuge, cesium chloride spontaneously formed a density gradient essential for Meselson and Stahl’s study of DNA. Because Meselson and Stahl subjected the cesium chloride solution to a large centrifugal force over many hours, the force gradually pushed the cesium chloride salt particles toward the bottom of the tube, which resulted in a higher concentration of cesium chloride farther from the rotor. Cesium Chloride never formed a complete pellet at the bottom of the test tube because of its natural tendency to diffuse, or re-dissolve, back into solution. The solution of the bottom of the tube became slightly denser than the solution at the top. The density of the cesium chloride solution increased along a gradient down the tube.

The cesium chloride density had a density range greater than the difference in densities between the heavy and light DNA that Meselson and Stahl aimed to separate. Therefore,
instead of centrifuging until the heavy DNA sank to the bottom of the container and the light DNA rose to the top of the container, Meselson and Stahl centrifuged the samples until the DNA suspended in separate parts of the solution and stopped moving at a point called equilibrium.

Density gradient centrifugation as developed by Meselson and Stahl employed a process called equilibrium sedimentation or isopycnic centrifugation. Equilibrium sedimentation is the process by which particles in a solution reach a point where they reach their isopycnic position and stop moving. Equilibrium sedimentation separates Meselson and Stahl’s centrifugation method from other types of density gradient centrifugation that do not allow particles to reach equilibrium where the density of the particles equals the density of the solution around them. The centrifugal force pushing the particles down equals the force of the solution pushing up, causing the particles to stop moving in the solution. The particles do not reach the bottom of the tube, as in other forms of centrifugation. Meselson and Stahl could determine the density of different DNA molecules based on where they reached equilibrium and stopped moving, because the molecules would stop when their density matched the density of the surrounding solution.

Meselson and Stahl conducted the Meselson-Stahl experiment in 1957 and 1958 with the successful density gradient centrifugation separation of DNA molecules of differing densities. To perform the technique, Meselson and Stahl first added one part lysed Escherichia coli bacteria cells containing heavy and light DNA to seven parts seven molar cesium chloride solution. To prevent the DNA molecules from breaking down in an acidic solution, the researchers maintained a constant level of pH six. Meselson and Stahl then centrifuged the samples for twenty-four hours at twenty-five degrees Celsius and 44,770 rotations per minute. Meselson and Stahl chose the speed of 44,770 rotations per minute for two reasons. First, if they spun the samples too fast, the samples would leak into the rotor chamber. Second, if they spun the samples too slow, equilibrium would take much longer to reach. The speed chosen, 44,770 rotations per minute, balanced the two. After centrifugation, Meselson and Stahl observed the formation of concentrated DNA in the form of dark bands across the sample container. The placement of the bands depended on the density of the DNA. Heavy and light DNA formed separate, distinct bands, with the heavier DNA further down the density gradient.

The particle bands formed during density gradient centrifugation have Gaussian or normal distributions that are symmetric about the mean average value. For the DNA band, the number of DNA molecules greater than the mean density equals the number of DNA molecules less than the mean density. The width of the band is inversely proportional to the centrifugal force. In other words, the faster the centrifuge spins, the sharper, narrower, and more defined the bands become. The bands are sharper because there is more centrifugal force to counteract diffusion. The width of the band relates inversely to the rate the density increases along the density gradient. The steeper the density gradient is, the sharper the band, which allow for more accurate measurements because the densities of the DNA molecules in the band are closer to the mean density. The width of the band relates to the molecular weight and mean density of the molecules in that band. Since Meselson and Stahl knew the approximate molecular weight of their DNA molecules, they used the bandwidth to calculate the densities of the DNA molecules they separated.

Density gradient centrifugation that utilizes equilibrium sedimentation in a cesium chloride solution assisted many scientists in many different experiments throughout the twentieth and
twenty-first centuries. Because the method enabled Meselson and Stahl to separate DNA molecules based on density alone, and not particle size or shape, the researchers were able to label parental DNA with denser elements and analyze the distribution of the heavy DNA over many replication cycles. Meselson and Stahl's findings allowed them to determine how DNA replicates. In 1960, at Caltech, Nobel laureates François Jacob and Sydney Brenner [9] used the same density gradient centrifugation method to show the presence of messenger RNA in cells, which scientists later determined to be an essential molecule for converting genetic information in DNA to expressed proteins. Scientists also later used density gradient centrifugation in a cesium solution to purify plasmids, circular pieces of bacterial DNA used for genetic engineering.

The type of density gradient centrifugation developed by Meselson and Stahl enabled scientists to analyze DNA and other nucleotides like RNA in a way they could not do previously. Unlike other forms of centrifugation, density gradient centrifugation in cesium salts separates molecules based on density only, rather than the size of the molecule or how fast that molecule travels in solution. Molecules like DNA remain undamaged in cesium chloride solutions, thereby enabling scientists to isolate, purify, and analyze those types of molecules. As of 2017, scientists still use density gradient centrifugation with cesium chloride to analyze DNA and RNA.

Sources

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