

“Control of Corneal Differentiation by Extracellular Materials” (1974), by Stephen Meier and Elizabeth D. Hay

In 1974, Elizabeth Dexter Hay and Stephen Meier in the US conducted an experiment that demonstrated that the extracellular matrix, the mesh-like network of proteins and carbohydrates found outside of cells in the body, interacted with cells and affected their behaviors. In the experiment, Hay and Meier removed the outermost layer of cells that line the front of the eye, called corneal epithelium, from developing chick embryos. Prior to their experiment, scientists observed that corneal epithelium produced collagen, the primary component of the extracellular matrix, which provides structural support to cells throughout the body. In their experiment, Hay and Meier confirmed that the lens capsule, a collagen-containing structure of the eye's extracellular matrix, induced the corneal epithelium to produce collagen. That result demonstrated that extracellular matrix interactions affect tissue development in developing embryos.

Hay and Meier researched cell and developmental biology together from 1972 to 1975 at Harvard Medical School in Boston, Massachusetts. During the 1960s, Hay studied collagen, which is the most common protein in the human body and comprises the majority of the extracellular matrix. In 1965, Hay had discovered that as eyes develop in bird embryos, collagen found underneath the cornea was being produced by corneal epithelium, a layer of sheet-like cells. According to Hay, many scientists at that time claimed that collagen was produced solely by fibroblasts, which are cells that synthesize components of the extracellular matrix, primarily collagen. Her experiment showed that the epithelial cells of the cornea, not just fibroblasts, could produce collagen.

Following her experiment, Hay began to question what factors caused the epithelial cells to produce collagen. Around the time of Hay's discovery, other researchers observed that a collagen containing portion of the eye that sits below the cornea, called the lens capsule, might affect how the corneal epithelial cells differentiates to synthesize collagen. Differentiation is the process during which embryonic cells become specific types of cells, such as skin cells or bone cells, which creates of new types of tissues during embryonic development. During the development of the cornea, corneal epithelial cells specialize, or differentiate, and synthesize collagen. Hay and James W. Dodson, a colleague of hers who studied the development of the cornea, performed an experiment in 1973 investigating the idea that the lens capsule affects the differentiation of the cornea.

In Hay and Dodson's experiment, they placed corneal epithelial cells on different substances to determine if the environments surrounding the cells affected their ability to produce collagen. When they placed the corneal epithelial cells on glass, they observed that the cells produced little collagen. But when they placed the corneal epithelial cells on the lens capsule, large amounts of collagen were produced. Furthermore, Hay and Dodson found that when they killed all of the living cells found on the lens capsule (leaving only extracellular matrix behind), the corneal epithelium continued to produce collagen. Hay hypothesized that extracellular matrix on the lens capsule affected the development of the cornea, specifically its production of collagen.

During the late 1960s and early 1970s, other researchers published suggested that collagen can induce various cells to differentiate, or become specific cell types. Meier, a postdoctoral researcher who studied cell differentiation, became a member of Hay's lab in 1972. In 1974, Hay and Meier designed an experiment to test whether or not the collagen found on the lens capsule somehow caused the cornea to develop.

Hay and Meier hypothesized that the collagen found in the lens capsule induced epithelial cells from the cornea to differentiate into eye cells and to synthesize more collagen. To test that hypothesis, they first removed a layer of corneal epithelium from the eye of a developing chicken embryo (*Gallus gallus domesticus*). They then placed the separated epithelial cells on top of various substrates, materials that enable cells to grow, and cultured them in laboratory glassware. Some of the substrates contained concentrations of collagen while others did not contain any collagen. Non-collagenous substrates included substances like glass, plastic, and fingernails. Because there are many different types of collagen found throughout the body, Hay and Meier tested various types of collagen, including the collagen found in the lens capsule. They also tested collagen found in tendons as well as collagen found in cartilage.

After placing the corneal epithelium on the various substrates, Hay and Meier then measured the amount of collagen produced under each condition. To identify and quantify the amount of newly synthesized collagen, Hay and Meier used radioactive chemicals. Prior to culturing the cornea epithelium, they added radioactive chemicals to the culture medium. As the cells made collagen, they would use those chemicals, but they would not use those chemicals for any other function. Consequently, Hay and Meier inferred that newly produced collagen contained the radioactive chemicals. They quantified the amount of collagen produced by measuring the radioactivity of the tissues, with higher radioactivity measurements indicating higher collagen production.

Hay and Meier found that when they placed corneal epithelium on collagenous substrates rather than non-collagenous substrates, the epithelial cells produced more collagen. That result indicated that the corneal epithelium cells differentiated, or became more specialized, when in the presence of collagen, such as that found in the lens capsule, and those differentiated cells produced more collagen. They observed similar results when they put the epithelial cells in environments with collagen from tendons and cartilage. That observation showed that collagen, including collagen that is not typically found in the lens capsule, caused the epithelial cells in the developing eye to differentiate into cornea cells.

Hay and Meier's experiment demonstrated that a component of the extracellular matrix, collagen, could affect the differentiation of epithelial cells. In 1981, the findings from their experiment were published in the book *Cell Biology of Extracellular Matrix*, which summarized research about extracellular matrix interactions. After Hay and Meier's 1974 experiment, scientists increasingly studied extracellular matrix interactions. Researchers found various receptors found on the outside of cells that physically interact with extracellular matrix components. Such interactions affect many aspects of a cell's behavior, including gene expression, migration, differentiation, and growth.

Sources

1. Dodson, James and Elizabeth Hay. "Secretion of Collagen in the Embryo. I. Morphology of the Collagenous Products Produced by Isolated Epithelia Grown by Frozen-killed Lens." *Journal of Cell Biology* 57 (1973): 190-213. <http://jcb.rupress.org/content/57/1/190.full.pdf+html> (Accessed May 30, 2016).
2. Hay, Elizabeth. *Cell Biology of the Extracellular Matrix*. New York City: Plenum Press, 1981.
3. Meier, Stephen, and Elizabeth Hay. "Control of Corneal Differentiation by Extracellular Materials. Collagen as a Promoter and Stabilizer of Epithelial Stroma Production." *Developmental Biology* 38 (1974): 249-70.
4. Trelstad, Robert L., Elizabeth D. Hay, and Jean-Paul Revel. "Cell Contact during Early Morphogenesis in the Chick Embryo." *Developmental Biology* 16 (1967): 78-106.
5. Trelstad, Robert L. "The Extracellular Matrix in Development and Regeneration: An Interview With Elizabeth D. Hay." *The International Journal of Developmental Biology* 48 (2004): 687-94.