Science Education Collection

Fate Mapping

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Abstract

Fate mapping is a technique used to understand how embryonic cells divide, differentiate, and migrate during development. In classic fate mapping experiments, cells in different areas of an embryo are labeled with a chemical dye and then tracked to determine which tissues or structures they form. Technological improvements now allow for individual cells to be marked and traced throughout embryonic development and adulthood.

This video reviews the concepts behind fate mapping, and then details a fate mapping protocol in zebrafish using photoactivatable fluorescent proteins. Finally, specific applications and modifications of this unique technique are discussed.

Transcript

Developmental biologists use fate mapping as a tool to trace cell lineages while an organism matures. This is done by labeling cells at an embryonic stage and then tracking them and their progeny throughout the organism's development. Fate mapping is also used to study cell migration and differentiation during development, as well as regeneration and repair during adulthood.

This video will provide an overview of fate mapping, explain a protocol used to generate a fate map in zebrafish, and show some ways in which this technique is currently being applied in labs.

Before jumping into the procedural details, let's discuss what a fate map is and how it's constructed.

In classical fate mapping experiments, scientists stained groups of cells in an early embryo, such as those in the gastrula stage, with a dye that would be passed on to all the descendants of these cells. After allowing the embryo to develop for a certain period of time, they viewed the stained cells in the more mature organism. The location of stained cells in the mature organism was then noted. Pooled results of several similar experiments allowed construction of a diagram known as a fate map.

Therefore, a fate map is an overall plan that outlines the fate of each part of an early embryo. These maps help scientists to determine things like which embryonic cells differentiate into which functional adult cells, and how they migrate and organize into mature structures.

Scientists have used many model organisms to create fate maps, including frogs, nematodes, fish, chicks, and mice. Some model organisms, such as the zebrafish *Danio rerio*, have an additional advantage in this type of experiment. Since they are small and remain transparent for much of the developmental process, scientists can easily track cells by viewing the fish under a light microscope. Importantly, advances in cell labeling techniques now allow scientists to precisely mark single cells and trace them as the organism develops, which helps in the creation of an extremely detailed fate map.

Now that you have an idea about what fate maps are, let's discuss a protocol for fate mapping in zebrafish that uses photoactivation. This relatively new approach depends on photoactivatable proteins. These are special fluorescent proteins, which are "caged," meaning they are held in a specific conformation to prevent fluorescence. An application of a controlled laser pulse causes a conformational change, referred to as "uncaging," that results in visible fluorescence.

In order to perform this experiment, these specialized caged proteins are first synthesized and then injected into one or two cell stage zebrafish embryos. Next, the embryos are allowed to mature to the desired developmental stage prior to photoactivation.

Then, to prepare the fish for photoactivation, the embryos are dechorionated to make the target tissue accessible. Next, they are mounted in an optically clear medium, such as low melting temperature agarose, which safely maintains them in a steady position. The samples are aligned to expose the area of interest, and mounted onto a laser-equipped microscope. A laser pulse is applied to the targeted area containing cells of interest, inducing photoactivation.

Following the laser treatment, embryos are carefully removed from the agarose and returned to their natural environment until the desired developmental stage is reached. In order to trace the photoactivated cells, embryos are again embedded in low melting temperature agarose, and the photoactivated cells can then be visualized and traced using direct fluorescence or immunostaining.

Now that you have an overall understanding of a fate mapping protocol, let's take a look at a few lab experiments that take advantage of this procedure.

In addition to studying embryonic development, fate mapping can be used to examine repair in mature systems. In this experiment, a specific cell subtype was ablated from a transgenic zebrafish retina. Scientists then traced genetically labeled resident adult stem cells to determine their fate following injury. Finally, image analysis was performed, which demonstrated activation of adult stem cells and subsequent tissue repair.

Scientists are also using similar protocols to understand the fates of transplanted stem cells. Here, genetically tagged human embryonic stem cells, or hESCs, were transplanted into an immunocompromised mouse model. The implanted cells were allowed to differentiate for 8-12 weeks, following which the resulting teratoma, which is a tumor that contains tissue from multiple germ layers, was harvested, fixed, and immunostained to determine the fate of implanted stem cells. This type of experiment helps scientists to confirm the *in vivo* differentiative potential of cultured stem cells.

As mentioned earlier, scientists perform fate mapping procedures in various model organisms, including mammals. In this particular study, scientists marked cells in a specific region of an early mouse embryo using inducible genetic approaches. This is done by administering an inducing agent to a pregnant mouse carrying genetically modified offspring. The labeled cells were tracked throughout later developmental stages, which helped scientists to determine their ultimate fate.

You've just watched JoVE's video on fate mapping. This video provided some insight into creating fate maps, reviewed a specific fate mapping protocol, and discussed some of the modifications and applications of this extremely useful technique. As always, thanks for watching!