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Laser Killing of Blastomeres in *Caenorhabditis elegans*

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INTRODUCTION

The *Caenorhabditis elegans* embryo is particularly amenable to microscopy and embryological studies because of its short developmental time, transparent shell, and nonpigmented cells. Within the embryo, contacts between cells often establish the polarization of neighboring cells. Blastomere isolation and recombination experiments have led to a wealth of understanding of the events in the four-cell *C. elegans* embryo. However, identifying individual blastomeres after isolation at stages past the four-cell stage is limited. In addition, removal of blastomeres from their native surroundings can interfere with many cell contacts besides the contacts of interest. An alternative approach for studying cell interactions within the *C. elegans* embryo is to use laser ablation of individual cells. Laser ablation can be used to kill one of two cells in contact with each other to understand what happens when a cell no longer signals to its neighbor. Additionally, killing a cell that is between two cells that will eventually contact each other can result in the corpse of the cell forming a steric barrier between the cells, preventing the contact. This protocol describes laser ablation of embryos mounted on an agar mount.

RELATED INFORMATION

A protocol for preparing live embryos for microscopy is described in **An Agar Mount for Observation of *Caenorhabditis elegans* Embryos** (Walston and Hardin 2010a). Protocols for collection and analysis of four-dimensional (4D) differential interference contrast (DIC) data can be found in **Acquisition of 4D DIC Microscopic Data to Determine Cell Contacts in *Caenorhabditis elegans* Embryos** (Walston and Hardin 2010b) and **Analysis of 4D DIC Microscopic Data to Determine Cell Contacts in *Caenorhabditis elegans* Embryos** (Walston and Hardin 2010c).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <!\>, and recipes for reagents marked with <R>. **Reagents** *C. elegans* embryos, mounted on an agar mount slide (see **An Agar Mount for Observation of *Caenorhabditis elegans* Embryos** [Walston and Hardin 2010a])

Equipment

Camera with monitor (attached to microscope)

This camera is used to follow the progress of laser killing.

Marker (if using a monitor with a glass screen; see Step 6)

Microscope (with DIC optics, ~60X–100X high-numerical aperture [NA] objective lens)

<!--Tunable dye laser (attached to microscope)

A common setup for ablation of *C. elegans* consists of a Micropoint tunable dye laser (Photonic Instruments), which includes a 337-nm nitrogen pumping laser and a dye cell filled with Coumarin 440 dye (5 mM in methanol). The intensity of the laser spot can be attenuated using a density gradient filter that slides to generate a spot of the appropriate diameter and intensity as judged by cracking of a coverslip (see Step 5).

METHOD

The total time needed is 15–30 min.

1. **Select embryos on an agar mount.**
2. **Place the embryos on the microscope, and locate them on the slide.**
3. **Focus the image on the top focal plane of the embryos.**
4. **Move the focus slightly above the embryos (essentially focusing on the coverslip of the slide), and move the microscope stage to remove the embryos from the field of view.**
5. **Activate a single pulse of the laser. If focused properly, the laser should crack or poke a small hole in the coverslip when pulsed.**
See Troubleshooting.
6. **Note on the monitor where the pulse cracked the coverslip.**
If using a monitor with a glass screen, marking the spot on the screen with a marker directly on the screen is convenient.
7. **Move the microscope stage back to the embryos, and focus on the nucleus of the blastomere to be targeted.**

The target nuclei should be in interphase of the cell cycle and should not be undergoing division.

8. **Lase the nucleus of the target cell with approximately 10 pulses/sec until charcoal buildup can begin to be seen within the nuclei, ~10–15 sec. See Troubleshooting.**
9. **Record embryonic development with 4D microscopy (see Acquisition of 4D DIC Microscopic Data to Determine Cell Contacts in *Caenorhabditis elegans* Embryos [Walston and Hardin 2010b] and Analysis of 4D DIC Microscopic Data to Determine Cell Contacts in *Caenorhabditis elegans* Embryos [Walston and Hardin 2010c]).**

See Troubleshooting.

TROUBLESHOOTING

Problem: No hole or crack appears in the coverslip when targeting the laser. [Step 5]

Solution: If no hole appears in the field of view, either the laser is not aimed properly down the objective within the field of view, or the focus is incorrect. The microscope must be focused on the coverslip. If the laser is properly positioned, slightly adjust the focus with the fine-focus control until the coverslip is in focus.

Problem: The embryo explodes when laser ablation is attempted. [Step 8]

Solution: If the focus is too close to the coverslip while targeting the nuclei within a particular embryo, the embryo will rupture as the laser cracks the coverslip. To prevent this, target focal planes of nuclei deeper within the embryo.

Problem: The lased cell fails to die and continues through development. [Step 9]

Solution: If the target nucleus is not lased sufficiently, it can recover, and the cell will resume mitotic divisions while the embryo progresses through development. Additionally, some cells progress through one final cell division before halting any future divisions. Depending on the particular nature and goals of the experiment, this may or may not be acceptable, and the cell should not be considered killed until cell divisions are halted. Many laser-killed blastomeres will display Brownian motion in which particles in the cytoplasm undergo rapid shaking movements following ablation. This should not be confused with cell division.

Problem: The entire embryo dies rather than an individual blastomere being killed. [Step 9]

Solution: Contrary to the previous problem of not lasing the nucleus long enough, if the entire embryo dies, it is usually caused by excessive lasing triggering embryonic arrest. Practice and experience help in determining the amount of lasing that will trigger cell death without killing the entire embryo.

DISCUSSION

In cases in which blastomere isolation is not feasible, an alternative is laser killing of particular blastomeres to understand the effects of those blastomeres on development or to create a barrier with the killed cell that prevents other cells from contacting each other. This technique does not require dissecting the embryo, and identification of individual blastomeres at the time of the killing and subsequent to the killing simply requires tracing the lineage of the remaining blastomeres. It can be conducted on embryos throughout development to study cells at a variety of stages of development. This technique can be conducted before 4D imaging or in the middle of collection of a 4D data set without removing the embryo from the microscope if it is set up with both the laser and the 4D imaging equipment.

REFERENCES

- Walston T, Hardin J. An agar mount for observation of *Caenorhabditis elegans* embryos. Cold Spring Harb Protoc. 2010a
- Walston T, Hardin J. Acquisition of 4D DIC microscopic data to determine cell contacts in *Caenorhabditis elegans* embryos. Cold Spring Harb Protoc. 2010b
- Walston T, Hardin J. Analysis of 4D DIC microscopic data to determine cell contacts in *Caenorhabditis elegans* embryos. Cold Spring Harb Protoc. 2010c