Labeling cells with photoconvertible fluorescent proteins in zebrafish

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Introduction:

Photoconvertible (aka photoswitchable, photoactivatable) fluorescent proteins exhibit fluorescence that can be modified by a light-induced reaction. In zebrafish, the exquisite optical accessibility of the embryo provides fantastic opportunities for labeling cells via photoconversion. A number of labs have taken advantage of photoconvertible proteins to track cells over time during embryonic development. To cite just a few specific examples (of course, this is not a comprehensive list of relevant studies):

- Photoconversion of individual cells has been used to track the dynamics of cell shape changes. Examples include studies in neurons (Sato et al., 2006) and in floor plate cells (Nakayama et al., 2012).
- Photoconversion of subpopulations of cells has been used to monitor cellular rearrangements during morphogenesis. Examples include studies of organ rotation during cardiac morphogenesis (Baker et al., 2008) and studies of cell division during morphogenesis of the neural tube (Tawk et al., 2007).
- Photoconversion of entire tissues has been used to birthdate cells. Examples include protocols for monitoring the timing of myocardial differentiation (de Pater et al., 2009) and the differentiation of trigeminal neurons (Caron et al., 2008).

Several types of photoconvertible proteins have been employed in zebrafish. The characteristics of three of the most commonly used proteins – Kaede, Dendra2, and KikGR1 – are listed in Table 1. They all share several features:

- Photoconversion is irreversible.
- There is no spontaneous conversion or cytotoxicity.
- The excitation/emission wavelengths for the original and photoconverted forms are clearly separable.
- The wavelength used for photoconversion does not overlap with the spectrum of wavelengths used for observation.

Table 1. Photoconvertible proteins commonly employed in zebrafish.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Kaede</th>
<th>Dendra2</th>
<th>KikGR1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>original</td>
<td>photoconverted</td>
<td>original</td>
</tr>
<tr>
<td>Excit./Emis. maxima (nm)</td>
<td>508/518</td>
<td>572/580</td>
<td>490/507</td>
</tr>
<tr>
<td>Oligomerization</td>
<td>Tetramer</td>
<td>Monomer</td>
<td>Tetramer</td>
</tr>
<tr>
<td>Isolated from…</td>
<td>the stony coral, Trachyphyllia geoffroyi</td>
<td>the octocoral, Dendronephthya sp.</td>
<td>the stony coral, Favia favus</td>
</tr>
<tr>
<td>Reference</td>
<td>Ando et al., 2002</td>
<td>Gurskaya et al., 2006</td>
<td>Tsutsui et al., 2005</td>
</tr>
</tbody>
</table>

Depending on the goals of a particular photoconversion experiment, there are choices to make regarding how to introduce the photoconvertible protein and the level of resolution
for photoconversion. As a source of photoconvertible protein, you might inject RNA or DNA encoding the photoconvertible protein or you might employ a stable transgene that drives expression of the photoconvertible protein. If your experiment requires restriction of photoconversion to a particular area, you might adjust the size of the pinhole on your compound microscope or you might select the region of interest using a confocal microscope. In the course, we will demonstrate methods for photoconversion on a compound microscope, using the following general protocol.

**Protocol:**

**Solutions needed:**

- 0.3X Danieau’s
- 3-4% methylcellulose
- Tricaine stock solution (use a final concentration between 100-200 ug/ml)

1. Prepare glass slides with bridges for mounting embryos. For embryos from gastrulation to somitogenesis stages, use three layers of No.1 cover slides to make bridges. For embryos 24 hpf and older, use two layers of No.1 cover slides. Use superglue to adhere slides together. Wearing gloves is recommended. Acetone (or nail polish remover) is helpful to clean up any stray superglue.

2. Dechorionate the embryos in 0.3X Danieau’s manually with forceps. (Egg water containing methylene blue can create background fluorescence and is therefore not recommended.) Embryos at gastrulation stages should be kept in a glass dish. Add Tricaine to the dish for embryos 18 hpf and older. Estimate the dilution of Tricaine needed to reach the desired final concentration, adding it gradually and until the embryos stop moving (and until the heart stops beating, if applicable).

3. Dilute some Tricaine into an eppendorf tube containing 3-4% methylcellulose. Vortex the tube at maximal rpm for 30 sec. Spin down the tube at RT in an eppendorf centrifuge at the maximal speed for 1 minute.

4. Use a serological pipetter with a fire-polished glass pipette to pick up one embryo. Bring along only a minimal amount of Danieau’s in order to avoid too much dilution of the methylcellulose. Insert the tip of the glass pipette into the eppendorf tube containing methylcellulose, and then slowly release the embryo into the prepared methylcellulose. Caution: active pipetting is not recommended, especially for young embryos. Be patient and let gravity do the work. After embryos gradually sink into the methylcellulose, remove the glass pipette and release all of the remaining liquid in its tip. Re-insert the tip of the glass pipette into the methylcellulose and suck up a small amount of solution (about 3 mm length within the pipette). Then suck the embryo into the glass pipette and drop the embryo very carefully onto the glass slide between the bridges. The diameter of the drop should be about 8 mm (either too much or too little methylcellulose will make it difficult to position the embryo in the following steps). Put another smaller drop of methylcellulose (about 2-3 mm in diameter) on one side of the cover slip. Merge the two drops together carefully (this will help to avoid bubble formation) as you place the cover slip on the glass slide, hanging upon the bridges. As pictured below, the cover slip
should not be aligned in parallel with the glass slide. A 45 degree rotation is better for 
use in the following step of positioning your embryo.

(Embryo image adapted from Kimmel et al., 1995)

5. Position your embryo as desired. Using two fingertips (typically the index fingers of your left and right hands), place tiny amounts of pressure exactly above the position of each bridge. Use subtle motions to slide the cover slip around until you achieve the desired orientation of your embryo.

6. Place the slide on the observation stage of the compound microscope. Find the embryo in bright field. Use the green filter set to detect the original form of Kaede. Use the red filter set to confirm that photoconversion has not already occurred.

7. Switch to the DAPI filter set and perform the actual photoconversion (exciting!). (For localized photoconversion, adjust the size of the pinhole.) After exposure of the embryo to UV, examine the fluorescent signal using the red filter set to determine whether photoconversion has been achieved. Then switch back to the green filter set to determine whether photoconversion has been complete. Repeat the photoconversion until no green fluorescence remains. (Note that the level of UV exposure will need to be calibrated for each individual microscope. Prolonged UV exposure is harmful to the embryos. In the Yelon lab, we find that 30-60 seconds of exposure can achieve complete photoconversion in embryos carrying Tg(myl7:kaede) and is compatible with normal embryonic development.)

8. Recover your embryo. For a short experiment (in which you would analyze the embryos within 3-4 hours after photoconversion), your embryo may be fine developing on the slide if you keep the slide in a covered, humid container. For a longer experiment, your embryo should be transferred into normal 0.3X Danieau’s medium. Slide the glass slide into a glass dish filled with 0.3X Danieau’s; be sure to use a glass dish when
working with younger embryos. Caution: don’t drop or dip the slide into the dish; instead, slide it in carefully at an angle. The methylcellulose around the embryo will gradually dissolve into the 0.3X Danieau’s, and the solution should gradually lift up the cover slip. If not, swirl the dish very gently to help lift the cover slip. Using a fire-polished glass pipette, transfer your embryo to a 24-well plate (agarose-coated for younger embryos). As before, when transferring your embryo, pipetting is not recommended; be patient and let gravity do the work.

9. At a later timepoint, analyze your results, using the red filter set to view the photoconverted Kaede and the green filter set to view the original form of Kaede.

References:


