

Chloroplast photorelocation movement

Chloroplasts relocate in the cell in response to environmental light conditions. There are two types of response: accumulation movement under weak light in which chloroplasts move towards light, and avoidance movement under strong light in which they move away from the light. In higher plants, these responses are induced by blue light through phototropin 1 and 2. In some species, particularly in lower plants including ferns, mosses and green algae, red light is also effective in inducing these movements through phytochrome. Here, procedures are presented for inducing chloroplast photorelocation movements by partial cell irradiation with a microbeam. In principle, microbeam irradiation of a cell is not difficult and you already have a basic set-up for microbeam irradiation if you have an epifluorescence microscope.

Microbeam irradiator set-up

Procedures

Epifluorescence microscopes consist of two light paths, one for ordinal transmission microscopy and the other for fluorescence microscopy. The latter light path is already a path for microbeam irradiation of a cell. The light path includes a field diaphragm. When you close the diaphragm to a minimal position, you are already performing microbeam irradiation with excitation light of the specimen placed on the stage. After replacing some parts of an old epifluorescence microscope (Nikon TMD), we are using this “hand-made microbeam irradiator” for the analyses of chloroplast photomovement (Kadota et al., 2001).

1. Change the parts of epifluorescence unit. The unit consists of an excitation filter, a barrier filter and a dichroic mirror. Remove the barrier filter and keep it empty. Replace the excitation filter with an interference filter of your desired wavelength (for example, when you analyze red or blue light response, replace with an interference filter having 660 nm or 450 nm peak, respectively and no transmission in other wavelengths. In our case, we use a filter peaked at 663.2 nm with half band width of 32 nm for red light and a filter peaked at 450 nm with half band width of 36 nm for blue light.). Change the dichroic mirror to a neutral density filter of around 50% transmission (half mirror).

2. Change the Hg lamp to halogen lamp. Epifluorescence microscopes usually have an Hg lamp. This type of lamp emits too strong a light and has a discontinuous light spectrum along the wavelength. Change it to a 100-W halogen lamp (common light bulb for transmission microscopy).

3. Place infrared filter in the light path for transmission microscope. As the observation of cells under visible light interferes microbeam-induced chloroplast movement, insert an infrared light transmitting filter (IR85 Hoya Glass Works) in the light path, coupled with the

use of an infrared light sensitive video or digital camera. In the case of higher plants, blue light alone is effective in the chloroplast photorelocation. In such cases, it is possible to use red light for observation.

4. Check the microbeam size and position. Place a grass slide on the stage and focus on its surface under dim observation light. You can see the microbeam (image of field diaphragm) in the center of the view. Close the field diaphragm to a desired microbeam size. In our case, we get a minimal microbeam size of 20 μm in diameter using X40 objective.

Measurement of light intensity of microbeam

As the microbeam is small in size, it is not possible to measure light intensity using a regular light meter. We use a silicon photodiode for which the manufacturer provides a calibration factor of photoelectric output along wavelengths

Materials

Silicon photodiode (S1227-66BR, Hamamatsu Photonics), Photosensor amplifier (C2719, Hamamatsu Photonics)

Procedures

1. Place the photodiode on the stage, collect all of the light beam inside the light-sensitive surface of the diode, and measure the output.
2. Calculate the light intensity using the following equation.

$$I = A / (B_{(\lambda)} * C)$$

I, Light intensity, A, photosensor output; $B_{(\lambda)}$, calibration factor; C, microbeam area

3. Light intensity suitable for chloroplast photomovement is summarized in the table below.

Plant	Blue light		Red light	
	Accumulation	Avoidance	Accumulation	Avoidance
Arabidopsis	1 Wm^{-2} or lower	10 Wm^{-2} or higher	-	-
<i>Adiantum capillus-veneris</i> (fern)	1 Wm^{-2} or lower	10 Wm^{-2} or higher	100 Wm^{-2} or lower	600 Wm^{-2} or higher
<i>Physcomitrella patens</i> (moss)	30 Wm^{-2} or lower	300 Wm^{-2} or higher	1 Wm^{-2} or lower	30 Wm^{-2} or higher

Induction of chloroplast photorelocation movement by partial cell irradiation with a microbeam

Procedures

1. Place cells or tissues on the surface of agar medium (1 to 3 mL) solidified in a 35 mm Petri dish and cover with a piece of cellophane or cover slip. Seal the dish with Parafilm. In the case of Arabidopsis leaf, fill the inner airspace of the leaf with water by vacuum

infiltration before placing on the agar medium.

2. Place the dish on the stage of the microbeam irradiator and focus on the target cell under infrared observation light using X40 long working distance objective (we use an Olympus ULWD40 which has a working distance of more than 10 mm).

3. Adjust the beam position to an appropriate region (usually the center) of the target cell by moving the stage.

4. Open the shutter of the microbeam light path and begin irradiation. The photomovement of the chloroplasts is generally apparent after irradiation for 0.5 - 3 h.