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Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede

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Abstract

In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these techniques label large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell–cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

Keywords: Fluorescent protein; Kaede; Photoconversion; Organotypic slice culture; Electroporation; 3D-time-lapse imaging; Neocortical histogenesis; Neuronal progenitor; Cell migration

Introduction

The temporally and spatially restricted events of neuron production and migration during early cortical development are important steps in the establishment of the laminated structure of the cerebral cortex. Abnormalities in these events result in malformations of the cortex and in turn lead to brain function deficits. The mechanisms underlying neuronal migration and differentiation may consist of a combination of intrinsic cues and environmental factors. Thus, to gain insights into the potential mechanisms that drive normal morphogenetic events, it is crucial to visualize the behavior of individual cells and their progeny in the brain.

Fluorescent vital dyes (e.g., DiI) have a long history as cellular markers, in part because they are very easy to apply to cells; after co-incubation, cells will readily take up such dyes. However, it is difficult to apply these dyes to a single cell, and they are therefore most often used to track the fate of particular cell groups. In recent years, fluorescent proteins have also been used as cellular markers in the field of neuroscience. Fluorescent proteins are easier to handle, less toxic, and not as subject to decreased fluorescence as are chemical dyes as a result of internalization, cell division, or cell growth. Fluorescent protein remains in a cell and its progeny as long as the vector remains present, and the appropriate transcriptional elements drive the expression of fluorescent protein. Thus far, a large number of color and functional variations of fluorescent proteins have been developed (for a review, see Matz et al., 2002; Miyawaki, 2004). In order to introduce a gene into living tissue, electroporation, replication-incompetent viral vectors, lipofection, and gene gun approaches are available. In and exo-

Abbreviations: E, embryonic day; IZ, intermediate zone; MZ, marginal zone; SVZ, subventricular zone; VZ, ventricular zone.

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utero electroporation is one powerful tool that has been used to introduce such marker proteins into the fetal brain (Tabata and Nakajima, 2001). It is easier and less time-consuming to apply this method than it is to construct viral vectors and generate virions; moreover, electroporation is more efficient at introducing genes than are lipofection and gene gun techniques. However, when fluorescent protein is introduced into tissues by electroporation, it still remains impossible to distinguish between individual cells in the tissues, in particular those near the electroporation site, as the fluorescent protein cDNA is introduced into many cells simultaneously. Therefore, there remains the need to develop alternative methods to mark and monitor specific cells and their progeny within intact, living brain tissue.

In order to establish a method of precisely identifying individual cells in cortical slices, we used the photoconvertable fluorescent protein, Kaede. Kaede (the Japanese word for "maple tree") is a recently isolated protein from a stony coral, *Trachyphyllia geoffroyi*, which contains a green chromophore that is photoconverted to a red chromophore (Ando et al., 2002). This chromophore contains the tripeptide His^{62}–Tyr^{83}–Gly^{64}. UV radiation induces an unconventional cleavage within the Kaede protein between the amide nitrogen and the alpha carbon at His^{62} (Mizuno et al., 2003). We were able to photoconvert single cells derived from a Kaede-introduced neuronal progenitor and follow their movements individually in mouse embryonic brain slices. This method enabled us to clearly distinguish a reddened cell of interest from other green fluorescent-positive cells in 3-dimensional tissue samples during extended time-lapse imaging studies.

Materials and methods

Mice

Pregnant ICR mice (SLC Japan, Inc.) were housed in a controlled environment under a regulated 12-h light/dark cycle. The day when a vaginal plug was detected was counted as embryonic day 0.5 (E0.5).

DNA preparation

Kaede full-length cDNA in the pCS2 expression vector (Ando et al., 2002) was amplified and purified with the QIAGEN (Hilden, Germany) plasmid maxi kit.

Electroporation

The surgical procedures performed on the pregnant mice and the embryo manipulations exo-utero were carried out basically as previously described (Tabata and Nakajima, 2001). The present experiments conformed to the guidelines for animal experiments set by the Japan Neuroscience Society. On E12.5, pregnant mice were deeply anesthetized with sodium-pentobarbitone at 50 μg/g body weight. The Kaede expression vector was dissolved in PBS (5 μg/ml), and Fast Green (final concentration: 0.01%) was added to the plasmid solution. After the embryos were exposed, approximately 1–2 μl of plasmid solution was injected into the lateral ventricle of the telencephalon with a glass micropipette. The embryos were nipped by the tips of a tweezers-type electrode with a diameter of 5 mm (CUY650-P3; Tokiwa Science, Fukuoka, Japan). Then, five electronic pulses (20 V, 50 ms, at intervals of 950 ms) were administered to each embryo with an electroporator (CUY21E; Tokiwa Science). The embryos were carefully placed back into the abdominal cavity; care was taken to avoid damaging the placenta in order to allow the embryos to continue developing. Adequate amounts of Kaede protein for labeling the cells by electroporation were sufficiently expressed only after 12 h.

Organotypic slice cultures

Brain slices were prepared basically according to the method of Miyata and co-workers (2002). The embryos were removed on the next day (E13.5) of electroporation. The cerebral hemispheres were isolated in ice-cold oxygenated Dulbecco's modified Eagle's medium (DMEM), and the hemispheres were manually sliced at a thickness of 200–300 μm with handmade micro-knives. The incorporation of Kaede protein into the slices was confirmed using a fluorescence microscope under conditions of blue excitation. The slices were then transferred to a culture medium, i.e., DMEM/F12 medium lacking phenolred (Invitrogen, San Diego, CA), which was supplemented with insulin (25 μg/ml; Sigma, St. Louis, MO), transferrin (100 μg/ml; Sigma), progesterone (20 nM/ml; Sigma), sodium selenate (30 nM/ml; Sigma), putrescine (60 μM/ml; Sigma), epidermal growth factor (EGF; 10 ng/ml; PrePro TechEC, Rocky Hill, NJ), basic fibroblast growth factor (bFGF; 10 ng/ml; Prepro TeckEC), horse serum (5%; Invitrogen), and fetal calf serum (5%; Invitrogen). Selected slices were transferred with 50 to 100 μl of culture medium onto handmade 35-mm glass-bottom culture dishes. A round cover glass (Matsunami; 22 mm in diameter) was attached to the bottom of a perforated 35-mm dish (Iwaki, Chiba, Japan) with silicon cement (KE42; Shinetzu, Tokyo, Japan). Brain slices were embedded with collagen I gel reagents according to the manufacturer's instructions (Type I Collagen; Nitta Gelatin, Tokyo, Japan). After the gel had solidified, approximately 0.6 ml of pre-warmed culture medium was poured onto the gel, and the medium was spread over the entire dish surface, including the rim, leading to a surface tension-induced reduction in the amount of medium at the center of the gel containing the slices (Miyata et al., 2002). Then, the dish was placed on a microscope stage equipped with a microincubator (Olympus, Tokyo, Japan). The atmospheric conditions were maintained by a flow of premixed N_2 gas including 5% CO_2 and 40% O_2.

Imaging and photoconverting

Slices were imaged with a confocal microscope controlled by a Fluoview FV300 or FV500 scanning unit (Olympus, Tokyo, Japan), a diode pumped solid-state laser Sapphire (488–20 nm; Coherent Radiation, Palo Alto, CA), a green He/Ne laser, and a 405-nm blue-violet laser diode (DL-LS5005; Sanyo, Osaka,