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Iontophoretic injection of Lucifer yellow CH into zygotes and blastomeres of the hydroid *Hydractinia echinata*

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ABSTRACT: The dye Lucifer yellow CH was iontophoresed into recently fertilized eggs and early blastomeres of *Hydractinia echinata*. Iontophoresis was carried out on the stage of an inverted microscope in order to follow filling of the injected cells by short pulses of epifluorescent illumination. Lucifer yellow proved to be nontoxic and development in embryos with injected blastomeres proceeded normally. When zygotes were injected all the cells of the forming embryo contained dye. When one of the first two blastomeres was injected all the progeny of the injected cell also contained dye. Dye-coupling between injected and uninjected blastomeres did not occur in two-cell embryos nor between descendants of either line. Development of Lucifer-yellow-filled blastomeres or zygotes could be stopped by blue light irradiation. In a number of injected cells, the dye tended to accumulate forming brightly shining spots. The dye did not penetrate the nuclear envelope of injected cells.

INTRODUCTION

Lucifer yellow is soluble in the cytoplasm of cells. Due to its low molecular weight, this fluorescent dye passes gap junctions and thus indicates electrically coupled cells (Stewart, 1978; Bennet et al., 1978; Pochapin et al., 1983). The relevance of dye-coupling for cell determination during embryonic development is under current discussion (for further literature see Dorresteijn et al., 1983). The fluorescence emitted by Lucifer yellow is very bright, a fact that allows easy detection of injected cells and their progeny. All the mentioned advantages make Lucifer yellow an excellent marker for tracing individual cell lines in developing systems. The aim of the present study was to determine whether Lucifer yellow meets the mentioned requirements for a cell lineage marker in a hydrozoan embryonic system.

MATERIALS AND METHODS

Zygotes and early embryos

Hydractinia echinata colonies were maintained in the laboratory at the marine biological station of Helgoland, Germany. The relevant methods have been described by Spindler & Müller (1972). Zygotes or appropriate embryos were collected every morning from the culture dishes and used for the experiments after being washed three times in Millipore filtered seawater.

Preparation of Petri dishes

Plastic Petri dishes were prepared as shown in Figure 1. The coverslips were coated with 1.5 % agar Noble (Difco). Prior to injection zygotes or embryos were placed in holes of appropriate size which had been dug into the agar using fine glass needles.

Preparation of electrodes

Electrodes were pulled from 1.0 mm outer diameter glass capillary tubing with filament (Hilgenberg, Malsfeld, Germany) using a vertical electrode puller (Kopf Instruments, Tujunga, Ca., USA). The tip was backfilled with 5 % Lucifer yellow CH lithium salt (Sigma) in *A. bidest., the remainder of the capillary was filled with 0.5 M LiCl.* The electrode resistance ranged from 30 to 100 MΩ.

Iontophoresis

Plastic Petri dishes (Fig. 1) containing the desired embryos were placed on the stage of an inverted microscope (Zeiss IM 35) and fixed with strips of modelling clay. The loaded electrode was brought to the surface of the embryo using a micromanipulator (Brinkmann, Mannheim, Germany). After impaling the electrode in the desired cell, hyperpolarizing current (4–10 nA) was applied for 6–8 min with the help of a microelectrode amplifier (L/M-1, List, Darmstadt, Germany). During iontophoresis the intracellular DC potential was monitored. It ranged from −30 to −50 mV and stayed stable during the period of dye injection.

Observation of embryos

After iontophoretic dye injection the electrode was removed and the embryos were transferred to glass dishes containing antibiotic sea water (100 µg Penicillin and 100 µg Streptomycin per ml sea-water). The injected embryos were kept in the dark. The specimens were studied in vivo using a Zeiss IM 35 inverted microscope with epifluorescent illumination. Micrographs were taken on Ilford HP 5 Asa 400 (Figs 3–5) or on Kodak CF Asa 1000 (Fig. 2).

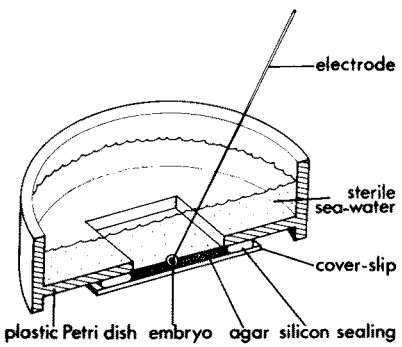

Fig. 1. Plastic Petri dish with glass coverslip forming part of the bottom. Hole in agar coating of coverslip contains embryo during iontophoresis

RESULTS

Zygotes of *Hydractinia echinata* injected with Lucifer yellow cleaved normally and produced normal planula larvae as compared with sham iontophoresed or untreated embryos. The fluorescent dye distributed equally between the blastomeres at each division of the embryo. When injected zygotes or embryos developing out of these zygotes were irradiated with blue light (450–490 nm) for more than 5 min, development stopped and no further cleavages took place. With shorter durations of blue light irradiation development was at least retarded and often stopped after one or two

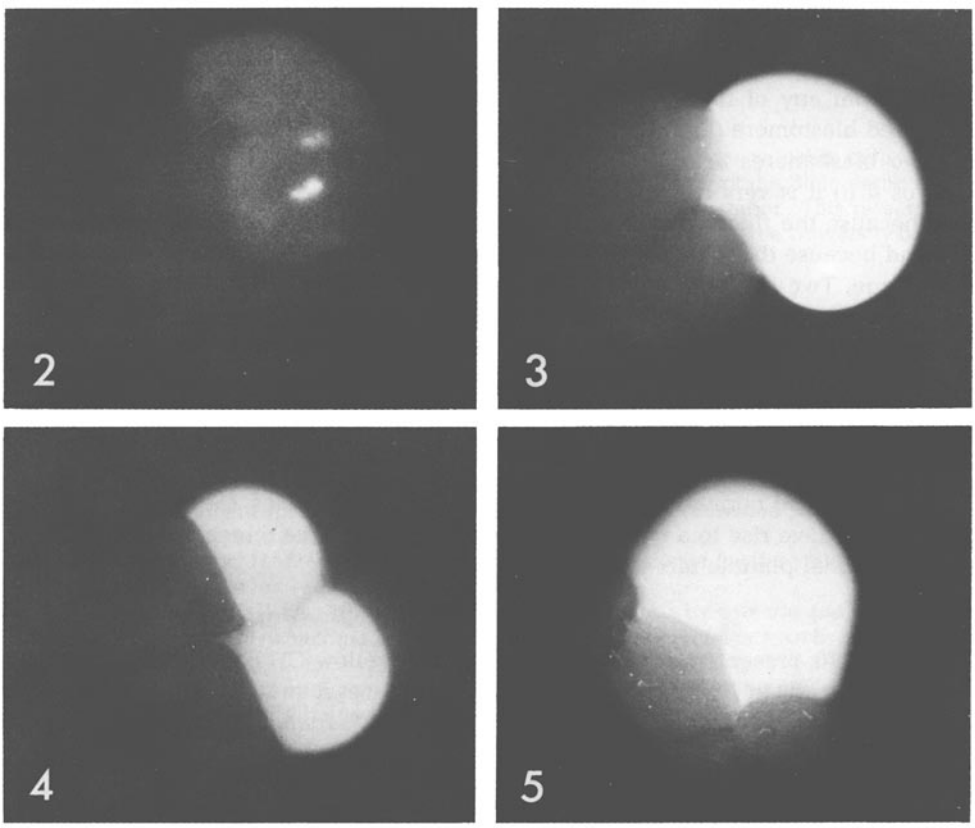

Fig. 2. 16-cell embryo with 4 fluorescent cells. 1 cell in the 4-cell embryo was injected with Lucifer yellow. Note overall weak fluorescence and shining spots in fluorescent blastomeres, x 165

Fig. 3. 2-cell embryo with one blastomere injected. Note that dye is confined to the injected blastomere. Note also that second cleavage synchronously begins in both cells, x 165

Fig. 4. 8-cell embryo with 4 fluorescent cells. 1 cell of a 2-cell embryo was injected. Note that dye did not diffuse into descendants of the uninjected blastomere, x 165

Fig. 5. 9-cell embryo. 1 blastomere of a 2-cell embryo was injected and subsequently irradiated with blue light (450–490 nm) for 6 min. The injected blastomere stopped cleavage while the uninjected blastomere continued to cleave undisturbed, x 165

subsequent cell divisions. In embryos developing out of injected zygotes the fluorescence emitted by each single blastomere was much less bright than it was in the zygote. It then became visible that the dye had not penetrated the nucleus, i.e. the nuclei of these cells were not fluorescent. In a number of injected cells the dye appeared to form aggregates or to accumulate at certain regions within the cells, which led to brightly shining spots (Fig. 2). Since no histological examinations were done it remains unsettled whether these accumulations of the dye are associated with distinct cell substructures. The tendency to form such accumulations increased with the number of cleavages following dye injection.

When one of the first two blastomeres was injected with Lucifer yellow, the dye remained in this cell and did not diffuse into the uninjected blastomere (Fig. 3). All the daughter cells of the injected blastomere also contained dye. There was no diffusion of the dye from any of the daughters of the injected cell to any of the progeny of the uninjected blastomere (Fig. 4). Obviously the cells of the two lines originating from the first two blastomeres are not electrically coupled during early development. In late embryos (8 h) it is very difficult to decide whether a cell belongs to one lineage or the other because the fluorescence emitted by a single cell has become very weak at that time and because the location of cells in late embryos does not appear to be related to cell lineage. Two-cell embryos with one cell injected developed normally as compared with sham iontophoresed or untreated controls. However, the resulting late embryos and planula larvae were not semi-fluorescent. The border-line between fluorescent and non-fluorescent cells was not identical in different embryos in which one of the first two blastomeres had been injected. Further cleavages of the injected blastomere in two-cell embryos or of the progeny of this cell could be prevented at any time by 5 min of blue light irradiation (Fig. 5). It thus was possible to evaluate the developmental capacity of the remaining and normally cleaving blastomeres. In such cases, the uninjected blastomere always gave rise to a normal embryo and planula larva which was about half the size of a normal planula larva.

DISCUSSION

The results presented demonstrate that Lucifer yellow CH can be used as a marker of embryonic cells in *Hydractinia echinata*. As in other systems (*Patella vulgata:* De Laat et al., 1980; *Arbacia punctulata:* Pochapin et al., 1983; *Psammechinus miliaris:* Pfannenstiel, unpubl. obs.) the fluorescent dye did not interfere with normal development of embryos. Dye-coupling between the first two blastomeres could not be observed. The same was true in *Arbacia* embryos (Pochapin et al., 1983). As in *Arbacia*, killing of the injected cell in two-cell embryos by blue light irradiation resulted in small but otherwise normal larvae. The question remains open, whether the lack of dye-coupling between the first two blastomeres in both sea urchin and hydrozoan is merely correlated to the capacity of these cells to produce a complete and normally shaped embryo or whether undetermined cells usually do not show dye-coupling. In *Patella*, dye-coupling becomes apparent shortly after the fifth cleavage (Dorresteijn et al., 1983). However, when cells were labelled shortly before the fifth cleavage they did not show dye-transfer upon subsequent development. The authors ascribe this feature to probable cytoplasmic binding of Lucifer yellow. The present author tends to believe that the observed accumulations of the dye in injected cells or their progeny also speaks in favor of

cytoplasmic binding of Lucifer yellow. So it would be interesting to inject one cell in a four-cell embryo of *Hydractinia* and see whether dye diffuses to its sibling cell or not. As judged from Figure 4b (Dorresteijn et al., 1983) the nuclei in the 64-cell *Patella* embryo were not penetrated by the dye. The same was true in *Hydractinia* embryos injected as zygotes or as two-cell embryos. The relevance of this finding is not clear. In neurons of various animal phyla, the perikarya and nuclei are very well stained by Lucifer yellow (Bicker, pers. comm.).

The set-up used for the present study can be recommended for further studies of that kind. It proved to be very helpful that the cells could be viewed by both normal and epifluorescent illumination while being injected. Thus, possible diffusion of the dye into neighbouring cells could be detected during iontophoresis. It also should be possible to follow the dynamics of dye diffusion in detail when injecting under permanent microscopic control.

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