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Review

Method for electroporation for the early chick embryo

Jun Hatakeyama and Kenji Shimamura*

Division of Morphogenesis, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan

In vitro whole-embryo culture of chick embryos, originally invented by New, has been widely used for studies of early embryogenesis. Here, a method for electroporation using the New culture and its derivatives is described, to achieve misexpression of exogenous gene in a temporally and spatially controlled manner in gastrulating chick embryos. Detailed information for the devices and procedures, and some experimental examples are presented.

Key words: chick gastrula, New culture, electroporation.

Introduction

Chick embryos have long been one of the favored model systems in the field of embryology and developmental biology. Recent advances in gene manipulation technologies (Muramatsu *et al*. 1997; Nakamura *et al*. 2004) make this model system even more attractive for developmental biologists (see review by Stern 2005). Thanks to its two dimensional geometry, ease of accessibility and observation, and well established fate maps, chick embryos have great advantages especially for studies at the early embryonic stages, such as the processes of gastrulation, neural induction, left-right patterning, etc. For such purposes, a whole embryo culture system, originally invented by New (New 1955), and its derivatives (Flamme 1987; Sundin & Eichele 1992; Stern 1993; Chapman *et al*. 2001) have been widely used.

Here we describe a method of electroporation for the early chick embryos using the *in vitro* whole-embryo culture. This method is applicable for some modified version of the New culture, by choosing an appropriate type of electrode. It can be applied for stage 4 to stage 8 embryos (Hamburger & Hamilton 1951), and they can be cultured up to stage 17 according to the culture methods. For a long-term study, the tissue of interest may be transplanted *in ovo* to the host embryo to let it survive for a longer period. This also allows precise positional control of the transgene expression in the host embryo. It should be noted that the younger embryos are the more sensitive to the electric stimuli in general, such that marked deformation of the embryos, even though they are alive, are frequently observed. Therefore, the voltage, pulse duration and numbers, and electrode distance, as well as DNA concentration should be optimized in each actual experimental condition. Cells in either the epiblast/ectoderm or the endoderm at relatively later stages (stage 6~) can be electroporated essentially in a similar way, except for the polarity of the electrodes and application of the DNA solution. As an example, we previously introduced exogenous genes broadly into the early anterior neural plate to demonstrate that specific responsiveness for the inductive signals was defined by the homeodomain transcription factors (Kobayashi *et al*. 2002).

Apparatus

1. Electrode set consisting of the upper and base electrodes (Fig. 1A,B; CUY701P2L and CUY701P2E, respectively; NEPA Gene, Japan). For cultures with a glass ring, the base electrode with a flat platform may be better (Fig. 1C) to use the ring as a chamber. The upper electrode is commonly used. Various sizes and shapes of the platinum electrodes are available from the manufacturer.

*Author to whom all correspondence should be addressed.
Email: simamura@kumamoto-u.ac.jp
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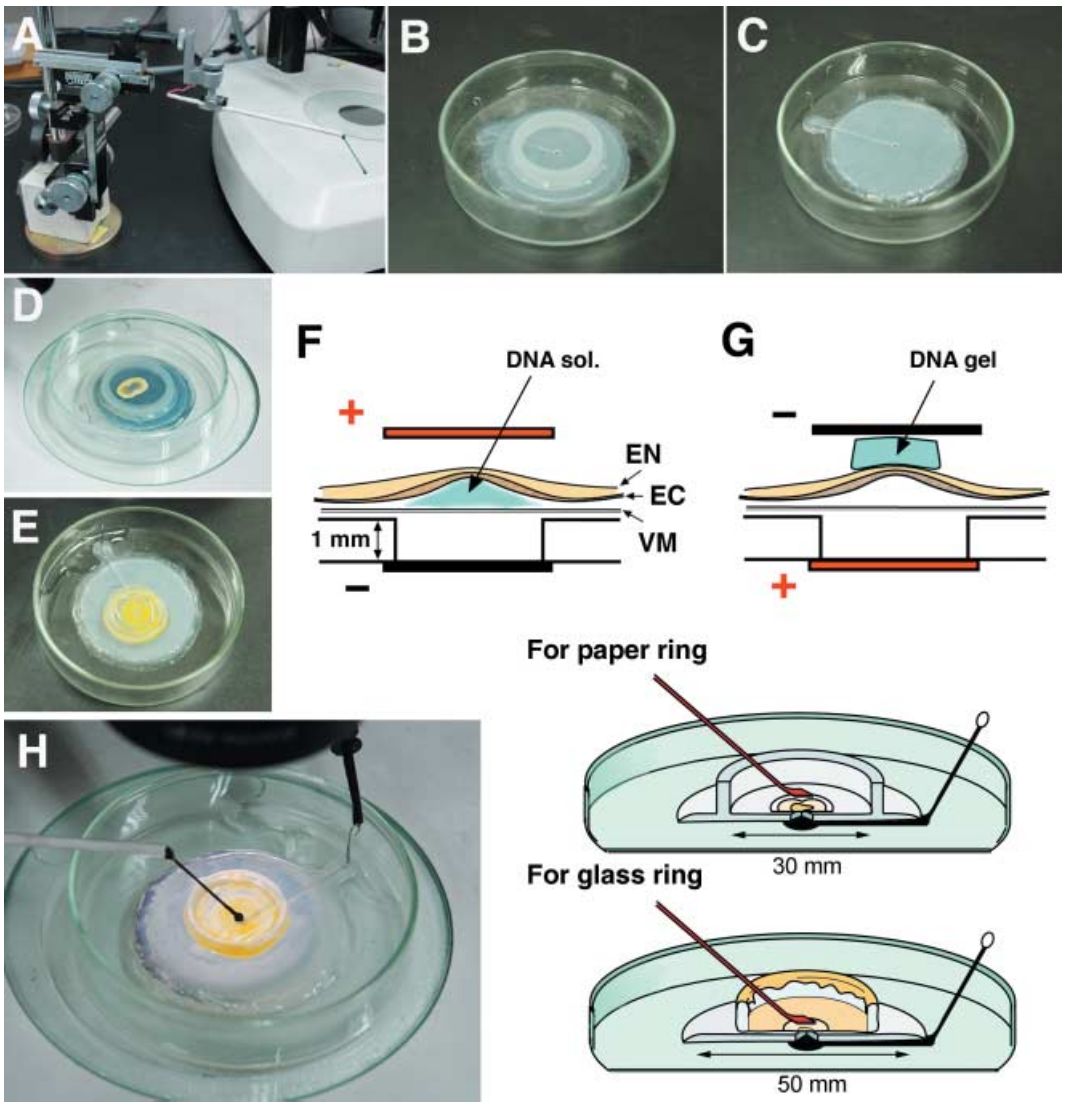

Fig. 1. Devices for electroporation for the early chick embryos. (A) Upper electrode with 2 mm-square platinum plate (CUY701P2L, NEPA Gene, Japan) set at the manipulator. (B) Base electrode with 2 mm^2 platinum plate for the culture with a paper ring (CUY701P2E, NEPA Gene, Japan). (C) Base electrode for the culture with a glass ring. The platform is simply flat. (D–H) Snap shots of the procedure. (F,G) Setting of the electrodes, embryo and DNA. Cross sectional views are illustrated. See text for details. EC, ectoderm; EN, endoderm; VM, vitelline membrane.

2. Square pulse generator (electroporator) (e.g. CUY21SC, NEPA Gene, Japan). It is ideal that the voltage can be set by 1 V, and the total electric current may be monitored for consistent operation.
3. Three dimensional mechanical manipulator with a stable base (e.g. Model MMN-3, Narishige, Japan). A scale at millimeter for the Z axis is desired.
4. Dissection microscope equipped with a transmitted light base.
5. Two pairs of fine forceps.
6. Two pairs of blunt forceps (for the culture with a glass ring).
7. Two pairs of sharp curved scissors for cutting the egg shell and the vitelline membrane, respectively.
8. Glass needle pulled out of a glass capillary (borosilicate, O.D. 1.0 mm, I.D. 0.75 mm) and silicone tubing with a mouse piece.
9. A fire-polished Pasteur pipette (for the culture with a glass ring).
10. Saline solution (Tyrod's, Ringer, phosphate buffered saline, etc.) with calcium and magnesium.
11. Incubator set at 39°C.
12. Humidified container incubated at 39°C.

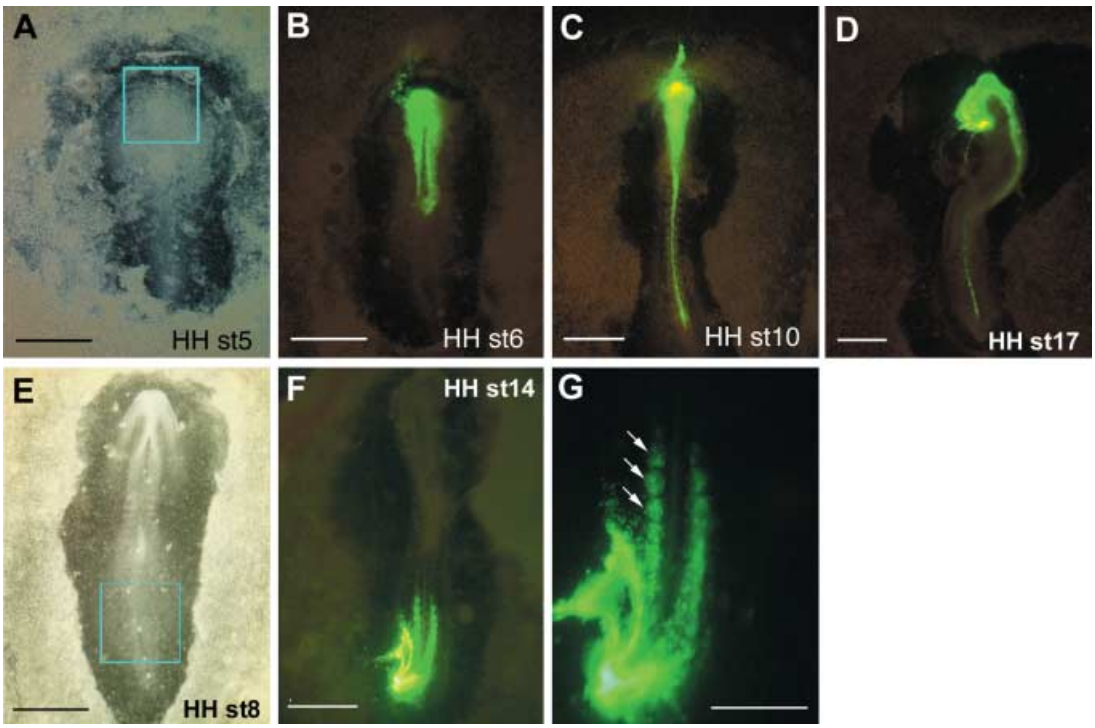

Fig. 2. Experimental examples. (A) A stage 5 chick embryo electroporated with pCAGGS-GFP plasmid *in vitro*. (B–D) The embryo under an epi-fluorescent dissecting microscope at stages 6, 10, and 17. The neural tube and some head ectoderm express green fluorescent protein (GFP). (E) pCAGGS-GFP plasmid was electroporated into the posterior portion of the primitive streak at stage 8. The targeted area is boxed. (F–G) GFP fluorescence is observed in the presomitic mesoderm and some somites (arrows) at presumptive stage 14. G is a magnification of F. Bars, 1 mm for A–F, 0.5 mm for G.

Procedure

1. Prepare the embryo with a glass or paper ring according to the modifications of the New culture.
2. Place the embryo on the platform of the base electrode (Fig. 1D,E; the ventral side up) which should be pre-rinsed with saline to avoid sticking. In the case of the paper ring, the base electrode chamber should be filled with saline in advance. Air bubbles in the electrode pocket should be cleared prior to embryo transfer.
3. Inject a DNA solution (~10 mg/mL) dyed with Fast Green® at the site of interest according to the fate maps. For the epiblast or ectodermal cells, inject the DNA solution between the vitelline membrane and the epiblastectoderm by penetrating the embryo from the ventral side carefully not piercing the vitelline membrane (Fig. 1F). For the endodermal cells, the DNA solution should be solidified with low melting agarose (~0.7% w/v). A small block of the agar/DNA is placed onto the endoderm layer (Fig. 1G). To introduce into the mesodermal cells, target the epiblast at the primitive streak according to the fate maps before they delaminate from the streak.
4. Set the embryo carefully so that the target field is precisely above the electrode plate. Then place the upper electrode attached to the manipulator right on top of the target field (Fig. 1H). Adjust the height of the upper electrode using the scale of the manipulator, so that the distance between the electrodes is consistent (usually 5 mm). Make sure that the upper electrode plate touches the liquid surface.
5. Check the polarity of the electrodes. For the epiblast/ectoderm targeting, the base electrode should be minus and the upper one should be plus (Fig. 1F). It should be reversed for the endoderm (Fig. 1G).
6. Deliver five square electric pulses of 10 volt, 50 millisecond duration with 100 millisecond intervals.
7. Remove the upper electrode, pick up the embryo with a glass or paper ring, and subject them to the culture process accordingly (for details, see the references). In the case of the glass ring method,

the saline within the ring should be carefully removed prior to transfer.

8. Keep the upper electrode wet. Remove the air bubbles generated around the base electrode pocket by flushing to prepare for the next round of operation. The saline in the electrode chamber should be changed periodically to clear the disposals.

Notes

1. The process of injection and electroporation should be done as quickly as possible so that the embryo does not come away from the paper ring.
2. The injected DNA solution is often swept away to the periphery of the neural folds due to tension generated by attaching to the paper ring. To avoid this, make the solution viscous, and create a space between the vitelline membrane and the ectoderm by gently lifting the embryo with the glass needle upon DNA injection.

Result examples

1. pCAGGS-GFP plasmid (Momose *et al*. 1999) was electroporated to the presumptive neural plate of stage 5 embryo. The targeted area is boxed. The expression of green fluorescent protein (GFP) was monitored during the course of culture. A broad region of the central nervous system (CNS) was introduced to the transgene (Fig. 2B–D).
2. The posterior region of the primitive streak was targeted at stage 8 and cultured for 24 h. The surface ectoderm, presomitic mesoderm, and some somites express GFP (Fig. 2F,G).

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