Introduction of Exogenous Genes into Chicken Embryos by Electroporation Using a Needle Type Electrode

Hiroki Furuta and Noboru Fujihara

Animal Resource Science Section, Division of Bioresource and Bioenvironmental Sciences, Graduate School Kyushu University, Fukuoka 812-8581, Japan

In this experiment, an exogenous gene, green fluorescent protein (GFP), was introduced into chicken blastodermal cells (stage X) just after oviposition using two types of electrode, parallel and needle, comparing embryonic survivability and subsequent development of embryos. The needle type electrode was prepared with 27-gauge needle of syringe which was covered with an insulating paint to isolate electricity. Electricity employed in this study was 10 v (needle type) and 200 v (parallel type) of pressure, 10 msec of pulse and 10 times of repeated intervals. The embryonic survivabilities were 65% for needle type and 53% for parallel one, respectively, though no significant (P>0.05) difference was observed between two types of electrode. The expression rates of GFP in embryonic tissues were 48% for needle type and 7% for parallel one, respectively, showing significant (P<0.01) difference between two types of electrode. In all of these treated embryos, GFP expression was mosaic manner for both types of electrode, indicating especially positive expression in embryonic tissues for needle type and in vitelline membrane for parallel one. Therefore, the present results suggest that the needle type of electrode might be superior to parallel one for introducing foreign genes into chicken embryos when used for electroporation.


Key words: gene introduction, chicken embryo, electroporation, needle type electrode, green fluorescent protein (GFP)

Introduction

In recent studies, electroporation method has been successfully employed to introduce foreign genes into animal cells, demonstrating that this technique would be better in skill and cost than micro-injection or some other kinds (Kino, 2000). The expression of introduced foreign genes has so far been reported to be less than 50 percent in case of chicken embryos (Muramatsu et al., 1996). On the other hand, efficient targeting of gene expression has been demonstrated in chicken embryos with electroporation (Momose et al., 1999).

Our previous experiments showed that the expression of exogenously introduced genes into chicken embryos varied considerably depending upon the loading condition of electricity in the electroporation method, suggesting a possibility of different effect of loading of electricity on the expression of foreign genes (Furuta et al., 2000).
On the contrary, successful transfer of foreign genes into chicken embryos by lipofection method has been reported when the gene was introduced into germinal crescent region (Eguma et al., 1999). Some of other successful results similar to this finding has also been obtained by the method of injecting exogenous genes into blastodermal cells of fertilized eggs just after oviposition (Inada et al., 1997; 1999; Furuta et al., 2000). In their experiments, the exogenous genes have also been observed at the germinal ridges and primordial germ cells (PGCs) of chickens. In the reports comparable to this, chimeric chickens have been produced by the transfer of chicken and quail PGCs or blastodermal cells (Petitte et al., 1990; Naito et al., 1994; Kagami et al., 1995; Ono et al., 1996; 1998; Furuta et al., 1999a, b; Yamaguchi et al., 2000).

The present experiments were carried out to introduce exogenous gene, the GFP, into blastodermal cells of freshly oviposited eggs with two types of electrode, needle and parallel ones.

**Materials and Methods**

**Recipient eggs and marker gene**

Freshly oviposited fertilized eggs (stage X) (Eyal-Giladi and Kochav, 1976) from White Leghorn hens were used as recipients. The GFP was used as a marker gene. Approximately 6.25 μg of the GFP was dissolved in 50 μl of Tris-EDTA buffer solution containing 10 mmol of Tris and 1 mmol of EDTA.

**Introduction of GFP**

A window of ca. 10 mm in diameter was opened on the sharp edge of recipient eggs. An aliquot (1.0 μl) of the GFP solution was injected into just central portion of blastoderm with fine glass pipette (G-1, Narishige, Tokyo, Japan) through vitelline membrane.

**Electrode and loading**

A needle type electrode was prepared with 27-gauge needle of syringe. The needle was covered with insulating paints to isolate electricity, leaving around 5.0 mm length from the top (Fig. 1). This electrode was inserted into egg yolk surrounding blastoderm as a negative charge.

A unilateral electrode of parallel type was placed on the vitelline membrane as positive charge. Loading of electricity for needle type was conducted as follows; 10 v of pressure, 50 msec of time (square pulse) and 50 msec of interval with 10 times repeating, respectively (CUY-21, BEX CO., LTD Japan).

The parallel type electrode was set on left and right sides of recipient blastoderm. Electric loading was conducted as follows; 200 v of pressure, 10 msec of time with 10 times repeating, respectively (Furuta et al., 2000). Immediately after loading, the window was covered with para-film.

**Incubation of treated eggs**

The treated eggs were incubated for 3 days at 38°C under 65-70% relative humidity till the embryos develop to stage 11-15 (Hamburger and Hamilton, 1951). At this stage, the eggs were broken to take out embryos and blood was collected from the embryonic
veins. The blood was smeared on slide glasses for air drying. The embryos and some tissues surrounding the embryos were rinsed with PBS.

Detection of GFP

Expression of GFP was examined in the PGCs from blood and embryos using fluorescent microscope (Nikon Opt. Co., Tokyo, Japan) and with MZ12 GFP Pulse fluorescence attachment (Leica Co., Tokyo, Japan).

Statistical analysis

Significant differences were determined in embryonic livability, expression of GFP and two types of electrode by Chi Square test.

Results

The needle type of electrode for electroporation led to slightly higher survivability and delayed embryonic development compared with parallel type (Table 1), though no

<table>
<thead>
<tr>
<th>Type of electrode</th>
<th>No. of eggs incubated</th>
<th>No. of eggs survived</th>
<th>No. of eggs with delayed development*</th>
<th>GFP expressed in the recipient eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of eggs</td>
</tr>
<tr>
<td>Needle</td>
<td>74</td>
<td>48 (64.9)</td>
<td>34 (70.8)</td>
<td>23 (47.9)*</td>
</tr>
<tr>
<td>Parallel</td>
<td>55</td>
<td>29 (52.7)</td>
<td>20 (68.9)</td>
<td>2 (6.9)*</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>13 (72.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Parallel type of electrode was used according to commercial one.
Figures in parentheses indicate percentage of survived embryos.
Percentage in the same column with different superscript are statistically different (P<0.01).
GFP: Green fluorescent protein

* Compared with developmental stage of control embryos at the same incubate time.
significant (P > 0.05) differences were observed between two types of electrode.

Gene expression rates of injected GFP were also higher in needle type electrode than in parallel type one, indicating significant (P < 0.01) difference between two types (Table 1). The use of needle type electrode resulted in higher expression of GFP in embryonic tissues and PGCs from the treated embryos (Fig. 2, 3). On the contrary, parallel type electrode induced restricted expression of the GFP in the area of vitelline membrane (Fig. 2). In the present studies, the expression of GFP gene was mosaic manner which mainly appeared in the vitelline membranes and embryonic tissues (Fig. 2).

Discussion

First of all, electric loading condition used in this experiment was the same one as employed in our previous method, since this condition led to best successful results for introducing exogenous gene into blastoderm in the chicken with parallel type of electrode (FURUTA et al., 2000).

The present experiments suggest that electroporation method for introducing foreign genes into chicken embryos brought about some kind of delayed embryonic development and higher incidence of embryonic mortality, though this technique has so far been reported to be easy method for transferring exogenous genes into chicken eggs (KINO, 2000). The previous experiments, therefore, the introduction of foreign genes into chicken embryos at stage 11–12 by electroporation led to around 50% of livability and gene expression (MURAMATSU et al., 1996). In our experiments, however, gene introduction into freshly oviposited eggs (stage X) with parallel type of electrode

Fig. 2. Expression of GFP gene in chicken embryos treated with needle type electrode.
A: Vitelline membrane
B: Abdominal cavity
resulted in lower rates of livability and gene expression compared with previous ones (Furuta et al., 2000). These results suggest a possibility of improving electroporation method to introduce foreign genes into chicken eggs.

Most interesting result obtained from this study was that the embryos treated with parallel type of electrode indicated typical gene expression on the site of vitelline membrane. However, delayed embryonic development after the treatment of eggs with parallel type of electrode have already been demonstrated (Furuta et al., 2000).

Based on the present works, then, the needle type electrode might be more effective for inducing higher rate of gene transfer into chicken eggs (stage x) than the parallel type one when electroporation method is employed.

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References


針状電極を用いた鶏初期胚への外来遺伝子導入

古田洋樹・藤原 昇

九州大学大学院農学研究院、福岡市 812-8581

平行型電極と針状電極を用いたエレクトロポレーション法により鶏初期胚にマーカー遺伝子 Green Fluorescent Protein (GFP) の導入を試みた。外来遺伝子を放卵直後の胚盤葉に注人した。今回の実験では 27 ゲージの注射針をマイナス極とした。処理後 3 日間孵卵を行い、鶏胚での GFP 遺伝子の発現を観察した。生存率は針状電極で約 65％（48/74）、平行型電極で約 53％（29/55）であり、両電極間に有意な差（P＞0.05）は認められなかった。一方、GFP 遺伝子の発現は針状電極で約 48％（23/48）、平行型電極で約 7％（2/29）であり、両電極間に有意差（P＜0.01）が認められた。GFP 遺伝子は両電極とももしくは数的な発現であった。

エレクトロポレーション法において、平行型電極より針状電極を用いた方が鶏初期胚への外来遺伝子導入はより効果的であると考えられる。

（家禽会誌、37：334-340, 2000）

キーワード：遺伝子導入，鶏胚，エレクトロポレーション，針状電極，GFP