BTX Applications

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dsRNA Transfection into Oocytes and Embryos

Efficient Delivery of dsRNA into Zona-Enclosed Mouse Oocytes and Preimplantatioin Embryos by Electroporation.

Double stranded RNA (dsRNA) has been shown to induce sequence-specific posttranscriptional gene silencing in a variety of organisms. Until recently, double stranded RNA interference (RNAi) molecules appeared ineffective in cultured mammalian cells and resulted in non-sequence specific suppression of translation. (1) By using longer dsRNA molecules of several hundred nucleotides, it was shown to induce sequence specific interference with gene expression in mammalian oocytes and preimplantation embryos. This use of longer RNAi molecules allows the study of gene expression during early mammalian development. (1) Standard microinjection techniques used to introduce dsRNA molecules into oocytes is effective to study the loss of gene function in single cells but is inefficient as a means of introducing dsRNA into more than a single embryo at a time. (1) Electroporation has been widely used as a method to introduce various molecules such as proteins, carbohydrates, viral particles, DNA, and mRNA into mammalian cells and living tissues, postimplatation embryos and male gametes and had potential to be an effective method to deliver dsRNA into oocytes or early embryos. (1) Optimized conditions were established in the ECM 2001(BTX Harvard Apparatus, Holliston MA) for electroporation using zona free maturing oocytes and 1 cell-embryos with rhodamine-conjugated dextran in a various microslide chambers (BTX Harvard Apparatus, Holliston MA) and various potential electroporation buffers.(1) From the various buffers tested including M2 medium, PBS (Phosphate buffered solution) and HBSS (Hepes buffered saline solution) results indicated that using HBSS results in higher survival rates, dextran uptake and embryo development. (1) Examining the efficiency between zona-free and zona-weakened maturing oocytes and 1-cell embryos indicated a higher rate of dextran uptake and increased embryo development for the zona-weakened embryos compared to embryos that were zona-free (See figure 1). (1) This approach was then applied to introduce dsRNA for *c-mos* into oocytes and green fluorescent protein (GFP) into transgenic GFP-expressing embryos at the 1- and 4-cell stages. (1) In both 1- and 4- cell embryos we observed sequence-specific interference with the expression of the target gene and a failure of oocytes to arrest at metaphase II and a loss in the green fluorescence of embryos by the morula or blastocyst stages. (1) Based on the finding of these experiments we believe that this method has the potential to introduce other macromolecules into oocytes and embryos in mice as well as a wide variety of mammalian species. (1)

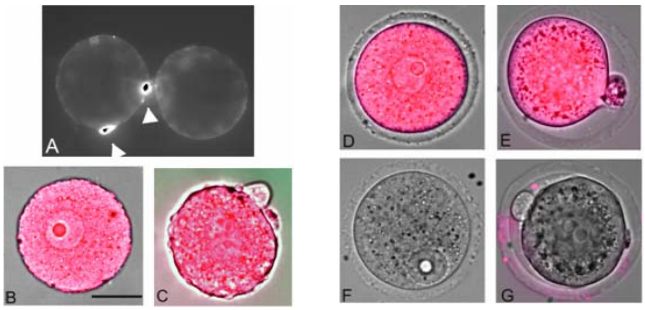

FIG. 1. Incorporation of rhodamine-conjugated dextran MW 3000 by oocytes and 1-cell stage embryos revealed by fluorescent microscopy. **(A)** Zona-free oocytes electroporated in M2 medium. Dextran uptake, revealed by fluorescence, is poor possibly because of precipitates (large ar-rows) produced in the medium during the proce-dure. Images B–G are merged fluorescent and DIC micrographs after electroporation with Dex-tran in HBS. **(B)** Zona-free oocyte. **(C)** Zona-free 1-cell stage embryo. **(D)** Zona-weakened oocyte. **(E)** Zona-weakened 1-cell stage embryo. **(F)** Zona intact (nonweakened) oocyte. **(G)** Zona intact (nonweakened) 1-cell stage embryo. Fluores-cence is present in the zona pellucida but not in the cytoplasm of the cell.

Protocol ECM 2001/ Microslide Chambers

Preparation:
Place groups of 20-30 intact GFP expressing embryos at 1-4 cell stage into a 100mm glass Petri dish filled with chilled HBS (Hepes buffered solution). Using a glass pasture pipette add approximately 20-50µl of acid Tyrode's solution to the embryos and immediately remove them from the solution. Wash the embryos in M2 media extensively to dilute the acid solution and allow embryos to sit in fresh M2 media for 30-60mins prior to experiment

Electrical setting:

Set Alignment Amplitude (AC):	NA
Set AC Time:	NA
Set DC Voltage:	20V
Set DC Pulse Width:	1-2msecSet **Number of**
Pulses:	3
Set Number of Series:	2
Chamber:	Flat Electrode 1mm gap (model 484)
Desired Field Strength:	0.2kV/cm

Electroporation Procedure:

Transfectant:	Dilute GFP dsRNA in chilled HBS for a final concentration of 20µg/ml and add 0.5-0.7µl of the dilution to the Flat electrode chamber.
Sample:	Place embryos into the chamber
Temperature:	Buffer for electroporation is chilled prior to experiment
Pulse:	Press Automatic **Start** to Activate Pulse Sequence
Plate:	Following electroporation wash the embryos through 37°C M2 media and place Into culture media and culture for up to 4 days until they reach morula/blastocyst stage

Results:
95% of the GFP expressing 1 cell embryos and 71% of the GFP expressing 4 cell embryos showed a decrease in the GFP expression

Reference:
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