General and Comparative Endocrinology 221 (2015) 165–172

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

# Transfection of isolated rainbow trout, *Oncorhynchus mykiss*, granulosa cells through chemical transfection and electroporation at 12 °C

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ARTICLE INFO

*Article history:*
Available online 6 May 2015

*Keywords:*
Granulosa
Rainbow trout
Transfection
Electroporation
Primary culture of cells

ABSTRACT

Over-expression or inhibition of gene expression can be efficiently used to analyse the functions and/or regulation of target genes. Modulation of gene expression can be achieved through transfection of exogenous nucleic acids into target cells. Such techniques require the development of specific protocols to transfect cell cultures with nucleic acids. The aim of this study was to develop a method of transfection suitable for rainbow trout granulosa cells in primary culture. After the isolation of rainbow trout granulosa cells, chemical transfection of cells with a fluorescent morpholino oligonucleotide (MO) was tested using FuGENE HD at 12 °C. Electroporation was also employed to transfect these cells with either a plasmid or MO. Transfection was more efficient using electroporation (with the following settings: 1200 V/40 ms/1 p) than chemical transfection, but electroporation by itself was deleterious, resulting in a decrease of the steroidogenic capacity of the cells, measured via estradiol production from its androgenic substrate. The disturbance of cell biology induced by the transfection method *per se* should be taken into account in data interpretation when investigating the effects of under- or over-expression of candidate genes.



## 1. Introduction

Nucleic acid transfection is now a common method used in a large variety of cellular models to study the functions of specific genes. The method chosen for the transfection of cells of interest therefore needs to be tested and selected to achieve a high transfection efficiency, but also to minimize deleterious effects on cell biology that could be induced by the method itself.

In teleostean fish, different approaches have been used to transfect cells (Table 1). Specifically, in rainbow trout, *Oncorhynchus mykiss*, an electroporation method has been developed to transfect the RTG-2 cell line (Chang et al., 2009; Ojima et al., 1999). However, chemical transfection can also be an efficient method for transfecting cell lines. Thus, plasmids were transfected into rainbow trout hepatoma cells (RTH-149) using Lipofectamine 2000 (Kling et al., 2013) and into RTG-2 cells using FuGENE HD, a lipid-based reagent (Ortega-Villaizan et al., 2011). Moreover, a calcium-phosphate transfection method was employed to efficiently transfer plasmids into RTG-2 cells (Collet and Secombes, 2001).

Most of these involve cell lines; few transfections have been performed in cells in primary culture. For example, in the European sea bass, *Dicentrarchus labrax*, ovarian follicular cells have been transfected with plasmids using FuGENE 6 (Crespo et al., 2013). Likewise, rainbow trout pronephros cells were transfected with plasmids using Turbofect, a cationic polymer (Ortega-Villaizan et al., 2012). Transfection of cells in primary culture is considered to be more difficult than transfection of cell lines (Hamm et al., 2002). However, an important requirement in using cell lines as a model is that they must resemble the normal physiology of cells *in vivo* (Brandon et al., 2003). To our knowledge, no study aimed at transfecting rainbow trout granulosa cells in primary culture has been performed previously, and the purpose of the present study was to develop such a transfection method. Both chemical transfection and electroporation were tested, using a fluorescent morpholino oligonucleotide (MO) together with FuGENE HD and either a fluorescent MO or a green fluorescent protein (GFP) reporter plasmid when performing electroporation.

*Abbreviations:* MO, morpholino oligonucleotide; GFP, green fluorescent protein; GSI, gonadosomatic index; RIA, radioimmunoassay; E2, estradiol-17β; Δ4, 4-Androstene-3,17-dione.

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http://dx.doi.org/10.1016/j.ygcen.2015.03.012
0016-6480/

**Table 1**
Example of fish cell transfection.

| Transfection method | Transfected cells | Species | Primary cells/cell line | Transfection reagents | Temperature | References |
|---|---|---|---|---|---|---|
| Electroporation | Embryonic cell line CHSE-214 | Chinook salmon *Oncorhynchus tshawytscha* | Cell line | | | Chi et al. (2012) |
| | Testicular germ cells and somatic cells | Japanese eel *Anguilla japonica* | Primary cell culture | | | Miura et al. (2007) |
| | Gonadal cells, RTG-2 | Rainbow trout *Oncorhynchus mykiss* | Cell line | | | Chang et al. (2009), Ojima et al. (1999) |
| Chemical transfection | Ctenopharyngodon idella kidneys cells, CIK | Grass carp *Ctenopharyngodon idella* | Cell line | FuGENE HD | Manufacturer's instructions | Yang et al. (2013) |
| | Epithelioma papulosum cyprinid carp cells, EPC | Common carp *Cyprinus carpio* | Cell line | FuGENE 6, Lipofectamine Rplus reagent | 28 °C | Lopez et al. (2001) |
| | | | | Lipofectamine 2000, Lipofectin, Perfect Lipid tfx | | |
| | | | | FuGENE 6 | 28 °C | Ruiz et al. (2008) |
| | Vertebra cell line, VSa13 | Seabream *Sparus aurata* | Cell line | Polyethylenimine | Room temperature | Braga et al. (2006) |
| | Hepatocellular carcinoma cell line, PLHC-1 | Top minnow *Poeciliopsis lucida* | Cell line | DMRIE-C | Room temperature | Chou et al. (2004) |
| | Fibroblast cells, CCF | Channel catfish *Ictalurus punctatus* | Cell line | SuperFect | NS | Zhang et al. (1998) |
| | Hepatocytes cells, Hepa-E1 | Eel | Cell line | Lipofectamine | 28 °C | Kitano et al. (2006) |
| | Embryo cells, HINAE cells | Japanese flounder *Paralichthys olivaceus* | Cell line | Lipofectamine | 20 °C | Simora et al. (2010) |
| | Gill cells, CSG | Murrel *Channa striatus* | Cell line | Lipofectamine 2000 | 28 °C | Abdul Majeed et al. (2014) |
| | Hepatoma cells, RTH-149 | Rainbow trout *Oncorhynchus mykiss* | Cell line | Lipofectamine 2000 | NS | Kling et al. (2013) |
| | Gonadal cells, RTG-2 | Rainbow trout *Oncorhynchus mykiss* | Cell line | FuGENE HD | 20 °C | Ortega-Villaizan et al. (2011) |
| | | | | Calcium-phosphate | 20 °C | Collet and Secombes (2001) |
| | Spleen and brain cells | Nile Tilapia *Oreochromis niloticus* | Primary cell culture | FuGENE 6 | 26 °C | Farahmand et al. (2003) |
| | Granulosa and thecal cells | Atlantic croaker *Micropogonias undulatus* | Primary cell culture | Lipofectamine 2000 | 24 °C | Dressing et al. (2010) |
| | Brain cells | European sea bass *Dicentrarchus labrax* | Primary cell culture | JetPEI, FuGENE 6 | 22 °C | Servili et al. (2009) |
| | Follicular cells | European sea bass *Dicentrarchus labrax* | Primary cell culture | Lipofectamine, FuGENE 6, calcium phosphate | 25 °C | Crespo et al. (2013) |

NS: non-specified.

## 2. Materials and methods

### 2.1. Ethical statements

Experiments were conducted according to the guiding principles for the use and care of laboratory animals in compliance with French (legislative decree No. 2013-118 of the French Ministry of Agriculture, Agri-Food Food and Forestry) and European (directive 2010/63/UE of the European Parliament and of the Council) regulations on the protection of animals used for scientific purposes. The animals were maintained in an officially recognized establishment (Approval No. B 35-238-6), and animal use was supervised by a French inter-agency local "Animal Care and Welfare Committee" (French national No. 7).

### 2.2. Animals

Rainbow trout (*O. mykiss*) females (2 years old; 1.0–1.5 kg body weight) were obtained from an experimental fish farm (PEIMA, Sizun, France) of INRA (the French National Institute for Agricultural Research). The fish were held in a recirculated water system at 12 °C under the natural photoperiod (Rennes, France). Vitellogenic or post-vitellogenic females were deeply anaesthetized in 2-phenoxyethanol (1 mL/L water) and subsequently euthanized (percussive blow to the head). Body (BW) and ovary (OW) weights were recorded, and the gonadosomatic index was calculated (GSI = (OW/BW) * 100).

### 2.3. Isolation and culture of trout granulosa cells

All work was performed under sterile conditions using a laminar flow hood in a thermostatically controlled room at 12–16 °C. The ovaries of the trout were quickly collected and immersed in trout mineral medium [TMM: 132 mM, NaCl, 3.1 mM KCl, 1 mM $MgSO_4$, 1 mM $MgCl_2$, 3.4 mM $CaCl_2$, 10 mM HEPES (Jalabert et al., 1973) at pH 8 and pO 300 mOsm/kg]. Ovarian follicles were separated from the rest of the ovary in TMM using tweezers. The isolated follicles were incubated with collagenase H (0.4 UI/mL) (Roche Diagnostics