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ELECTROPORATION OF snoRNA IN *Giardia lamblia*

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Abstract

Electroporation is a significant increase in the permeability of the cell membrane caused by an externally applied electrical field. It is used in molecular biology for the introduction of various micromolecular substances into a cell. Compared to other biochemical techniques, electroporation is simple, easily applicable and very efficient. This study reports the electroporation of *invitro* transcribed giardial snoRNA into *Giardia lamblia* by varying the number of pulsed under a constant electric field and its visualisation using a confocal microscope.

***Keywords**: Giardial lamblia*, electroporation, snoRNA.

1. Introduction

Several biochemical methods have been developed for transferring genes into cells which includes incubation of recipient cells with co-precipitates of DNA and Ca-phosphate (Graham and van der Eb, 1973), microinjection of genes into the nucleus of the recipient cell (Diacumakos, 1973), use of viral vectors (Hamer and Leder, 1979: Mulligan et al., 1979) and use of liposomes as vehicles of gene transfer (Fraley et al., 1980; Wong et al., 1980; Schafer-Ridder et al., 1982).

A biological membrane is a co-operatively stabilized organisation of lipids and proteins containing locally limited structural defects. These local disorders are the candidates for the onset of further electric field induced perturbations leading to permeation sites for enhanced material exchange (Neumann et al., 1982). The membranes, transiently, increases their permeability under the action of short electric field pulses (Neumann et al., 1972; Zimmerman et al., 1973) when a certain threshold value of the field strength is exceeded (Neumann et al., 1972). This increase in permeability leads to a transient exchange of matter across the perturbed membrane structures. The electric field effect on transport is clearly a membrane phenomenon (Sale and Hamilton, 1968; Rosenheck et al., 1975; Linder et al., 1977).

The process of electroporation is often used for transformation of cells. A high voltage is applied across a distance of few millimetre to produce reversible pores in the cell through which the uptake takes place. The cells are then carefully handled for their survival. Electroporation is 10 times more effective than that of chemical transformation (Neumann et al., 1982; Sugar et al., 1984).

Giardia lamblia is a gastrointestinal flagellated protozoan parasite causing giardiasis to an estimated 300 million people worldwide (Karmakar et al., 2013). Although the organism lacks some of the eukaryotic organelles, previous studies have reported the presence of ribosomal RNA (rRNA) processing system in *Giardia* (Karmakar et al., 2013;). Small nucleolar RNAs (snoRNAs) are a group of small non-coding RNAs (sncRNAs) that are known to be involved in the processing of pre rRNA or any other aspect of ribosome biogenesis (Ghosh et al., 2013).

In this study we describe the process of electroporating *invitro* transcribed giardial snoRNA into the organism by using different number of pulses under a constant electric field.

2. Materials and Method

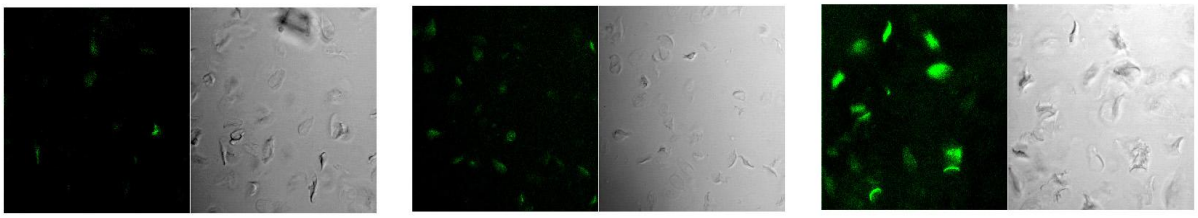
RNA J is a U14 class of snoRNA of *Giardia* characterised by the presence of A and B conserved domains, along with the C and D conserved sequence (Ghosh et al., 2013). The RNA was cloned in pGEM 4z vector as described previously (Ghosh et al., 2013). The recombinant plasmids were isolated, single digested and linearized with EcoR I restriction enzyme (NEB) and used for *invitro* transcription (IVT) by using T7 Maxiscript kit (Ambion), following the manufacturer's protocol. The RNA were tagged with Fluorescein 12 UTP (Roche) for easy detection inside the cell by the help of a confocal microscope (LSM 510 Meta, Zeiss). This transcribed RNA was purified by using mini Quick Spin Columns (Roche) according to manufacturer's protocol for removing the excess and unincorporated Fluorescein 12 UTP.

Electroporation was carried out in Electro Cell Manipulator ECM 2001 (BTX) electroporator. *Giardia* trophozoites grown axenically in modified TYIS 33 medium were subjected to cold shock by keeping the culture tubes in ice for 10 mins and the cells were collected by centrifugation at 1600 rpm for 10 mins. Cells were then washed with sterile PBS (NaCl 137mM, KCl 2.7 mM, Na_2HPO_4 10mM, KH_2PO_4 2.0 mM) for 3 times and resuspended in it at a concentration of 10^7 cells/ml.

0.8 ml of this cell suspension was taken in a 2 mm gap cuvette, *invitro* transcribed RNA J was added, and incubated on ice for 10 mins. This was then electroporated at 2.5 kV for a pulse length of 20 ms. Pulses of 8, 10 and 12 were applied to determine their optimum number for maximum electroporation. Electroporated cells were then incubated on ice for 15 mins, washed with PBS to remove excess RNA and observed under a confocal microscope.

3. Results

At a potential difference of 2.5 kV across a distance of 2mm, it was observed that snoRNA incorporation in *Giardia lamblia* depended on and is directly proportional to the number of pulses (Fig. 1). As is evident from the figure, with 8 pulses almost negligible amount of RNA could be imported into the cells. When the number of pulses is increased to 10, a higher amount of RNA is electroporated, but many cells are visible which has no electroporated RNA inside them. When the number of pulses is increased to 12, it is evident that the proportion of cells containg the electroporated RNA is the highest compared to others. Almost all the cells contain the electroporated RNA.



A B C

Figure 1. Confocal Microscopic pictures showing *Giardia lamblia* with electroporated RNA J inside them. (A) shows the cells after 8 pulses; (B) shows the cells after 10 pulses; (C) shows the cells after 12 pulses.

4. Discussion

As has been reported in previous studies, it is desirable to use a minimum electrical field for electroporation because an unnecessary increase in the electric field compromises the viability of the cells (Ivorra *et al*.). From this study it is evident that for a constant potential difference of 2.5 kV across a distance of 2mm, 12 pulses of 20ms each is the minimum number required for electroporating sufficient snoRNA into *Giardia lamblia*. Pulses lesser than that is unable to incorporate sufficient RNA into the cells. Electroporation is an important tool for molecular biology and use of this technique for introductions of foreign molecules in *Giardia* could help us understand the cell biology of this organism.

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