Transfection of mouse C2C12 myoblasts with Metafectene Pro

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Introduction

Nebulin is a giant structural protein of the sarcomeric thin filaments. It is expressed in vertebrate skeletal muscle where it comprises approximately 3% of the myofibrillar proteins. A lower expression of nebulin has been detected in cardiac muscle. Mutations in the nebulin gene (NEB) are the most common cause of autosomal recessive nemaline myopathy. Nebulin is encoded by one gene which undergoes extensive alternative splicing resulting in isoforms ranging from 600 to 900 kilodalton. The nebulin gene (NEB) is 249 kb in size, and comprises 183 exons. Based on the nebulin protein organization and our observations of the splicing pattern of NEB in different muscles, we predict the theoretical number of different nebulin isoforms to be 3388.

We are investigating nebulin pre-mRNA splicing using different nebulin minigene constructs. The expression vectors are transfected into C2C12 mouse myoblasts, and the splicing is assessed by RT-PCR, gel electrophoresis and sequencing.

Materials and methods

Metafectene Pro was obtained from Biontex Laboratories GmbH (Munich, Germany). Mouse myoblast cell line C2C12 (ATCC, and CRL-1772) was purchased from ATCC (Manassas, VA, USA). The cells were maintained in DMEM (BioWhittaker, Cambrex, Belgium) supplemented with 10% FBS (Gibco), 1% glutamax (Gibco) and gentamycin (5 µg/ml, INC biomedical). The C2C12 cells were incubated at 37°C in a CO_2-incubator. Nebulin minigenes were constructed as described in Lehtokari et al. (2006).

Experimental

For transfection $1x10^5$ – $1.5x10^5$ C2C12 cells were seeded in each well of a 6-well cell culture plate (Nunc, Roskilde, Denmark), in DMEM supplemented with 10% FBS, glutamax and gentamycin, 2 ml/well. The following Metafectene Pro:DNA ratios were tested: 2:1, 3:1, 4:1. Metafectene Pro and DNA were diluted in PBS. Three different transfection protocols were tested.

Protocol 1.

The cells were grown for 24 hours before transfection. The growth medium was changed to DMEM without FBS and gentamycin, 1.8 ml/well. The following transfection solutions were prepared:

Solution A: 2 µg DNA in 100 µl PBS (altogether 6 vials for one 6-well plate)

Solution B: 4 µl, 6 µl and 8 µl Metafectene Pro in 100 µl PBS (2 vials of each)

Solutions A and B were combined (solution A was added into solution B) and incubated for 15 min at room temperature. The transfection solution was added dropwise to the cell culture wells (200 µl/well). The cells were incubated for approximately 24 hours at 37°C in a CO_2-incubator before RNA extraction.

Protocol 2.

$1x10^5$ – $1.5x10^5$ C2C12 cells were seeded in each well of a 6-well cell culture plate in DMEM without FBS and gentamycin, 2 ml/well. The cells were incubated for approximately 2 – 3 hours at 37°C in a CO_2-incubator before transfection. The Metafectene Pro:DNA ratio 2:1 was applied. Solution A contained 2 µg DNA in 100 µl PBS and

Solution B contained 4 μl Metafectene Pro in 100 μl PBS. Solutions A and B were combined (solution A was added into solution B) and incubated for 15 min at room temperature. The transfection solution was added dropwise to the cell culture wells (200 μl/well). The cells were incubated for approximately 24 hours at 37°C in a CO_2-incubator before RNA extraction.

Protocol 3.

Approximately 10^5 C2C12 cells were seeded in each well (6-well-plates) and the cells were incubated at 37°C in a CO_2-incubator over night. The Metafectene Pro:DNA ratio 2:1 was applied as follows:

Solution A: 4 μg DNA in 100 μl PBS

Solution B: 8 μl Metafectene Pro in 100 μl PBS

Solutions A and B were combined (solution A was added into solution B) and incubated for 15 min at room temperature. Meanwhile, the C2C12 cells were washed with PBS and the medium was changed to DMEM without FBS and gentamycin, 1.8 ml/well. The transfection solution was added dropwise to the cell culture wells (200 μl/well). The cells were incubated at 37°C in a CO_2-incubator for 2 hours, where after the medium was changed to DMEM supplemented with 10% FBS, glutamax and gentamycin, 2 ml/well. The cells were cultured approximately 20 hours at 37°C in a CO_2-incubator before RNA extraction.

RNA extraction, RT-PCR and sequencing were done as described in Lehtokari et al. (2006).

Results and discussion

The Metafecten Pro:DNA ratio 2:1 turned out to be the most effective one. Signs of toxicity (increased cell death) were observed in the cultures exposed to ratios 3:1 and 4:1. No differences in transfection efficiency was observed between protocols 1-3, they all work well, and sufficient RNA for RT-PCR was obtained from all cultures.

Summary

Metafectene Pro was highly effective in transfection mouse C2C12 myoblasts. The possibility to prepare the transfection solutions in PBS and the possibility to add the transfection complex to freshly seeded cells are clear advantages of Metafectene Pro compared to other transfection reagents.

References

Lehtokari, V-L., Pelin, K., Sandbacka, M., Ranta, S., Donner, K., Muntoni, F., Sewry, C., Angelini, C., Bushby, K., Van den Bergh, P., Iannaccone, S., Laing, N.G. & Wallgren-Pettersson, C. Identification of 45 novel mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. Human Mutation, 27:946-956, 2006.