RIBOPRO

APPLICATION NOTE 03

In contrast to plasmid transfection, mRNA yields uniform protein expression after only 3 hours.

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INTRODUCTION

The analysis of protein function through transient expression is commonplace in cell- and molecular biology. A general concern are non-physiological effects through overexpression which may initiate compensatory or non-endogenous pathways. The chance for compensatory pathways will increase with the time elapsing between transfection and observation. Moreover, highly heterogenous expression levels across the cell population may compromise biochemical analyses, when for example protein interactions are probed by co-immunoprecipitation. Thus, the most reliable results are to be expected if all cells show uniform expression levels. Here, we compared the kinetics and uniformity of expression for cells transfected either with eGFP mRNA or a plasmid coding for eGFP. For expression, plasmids need to enter the cell nucleus which can only occur for dividing cells. By comparison, mRNA can also be expressed in non-dividing cells.

METHODS

EGFP mRNA (RIBOPRO) or the pEGFPz.N1 plasmid were formulated with lipofectamine (Lipofectamine MesssengerMAX for mRNA and Lipofectamine 3000 for plasmid transfection) according to the manufacturer's specifications. One day prior to the experiment HeLa cells were seeded in Ibidi µ-slides at a density of 20,000 per well. The next day, cells were incubated with the mRNA and plasmid formulations (25ng/well for both). At the indicated time points, cells were imaged by confocal microscopy using a Leica SP8 HyD confocal microscopy using White Light Laser. Fluorescence was excited at 488 nm and detected between 500-550 nm using an HC PL Fluotar 20 x 0.5 N. A. dry lens. Laser intensities were adjusted to avoid saturation and enable detection of

fluorescence above background, whereas all other imaging parameters remained unchanged. Quantitative image analysis was performed using ImageJ.

RESULTS

We have shown before that mRNA expression yields excellent control over protein dose.1 Here, we were interested in the expression kinetics and homogeneity of expression for cells transfected with either eGFP mRNA or plasmid DNA. For this purpose, sub-confluent cells were transfected with mRNA or plasmid coding for eGFP and imaged by live cell confocal microscopy starting at 3 hours after transfection. At this early time point, for the mRNA-transfected cells, expression was already detected in most of the cells (Figure A, B). By comparison only very few plasmid-transfected cells showed protein expression. As a consequence, for the mRNA transfection the number of positive cells showed only limited further increase for the remainder of the experiment, whereas for the plasmid-transfected cells, this figure increased until 48 h (Figure B). With respect to expression levels, fluorescence of the plasmid-transfected cells increased over the whole time course of the experiment and exceeded the one for the mRNA transfected cells (Figure C). However, expression levels were more heterogenous than the ones for mRNA transfected cells. The persistent increase and heterogeneity were also apparent when the total cellular fluorescence over time was plotted (Figure D).

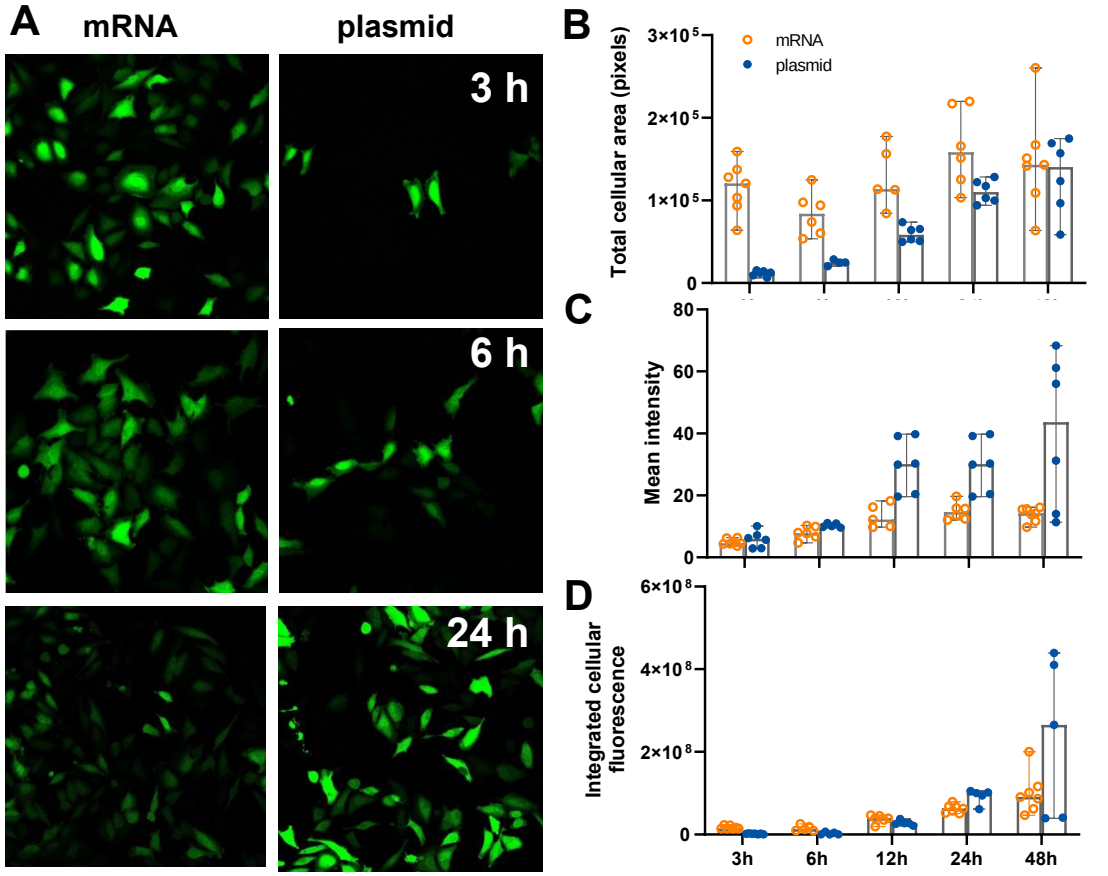

Figure: (A) Live-cell confocal microscopy of HeLa cells transfected either with eGFP mRNA or plasmid DNA. For each time point, the laser intensity was adjusted to enable detection of eGFP expressing cells and avoid saturation. Thus, at 24 hours eGFP expression in the mRNA-transfected cells was not lower than at 6 hours but only less in comparison to the plasmid-transfected cells. (B) Total surface area within a field of view positive for eGFP expression as a measure of eGFP expressing cells; (C) mean pixel intensity of cells showing eGFP expression above background levels. Differences in laser power between time points were corrected for by assuming a linear dependence of emission intensity on laser power; (D) integrated cellular fluorescence representing the total fluorescence above background in each field of view. In (B-D) each point represents one field of view, error bars are standard deviations of the mean.

CONCLUSIONS

Transfection with mRNA yielded uniform protein expression across the cell population already after 3 hours whereas for plasmid DNA only few cells showed high expression levels. For the plasmid-transfected samples, heterogeneity remained high over the full-time course of the experiment. The uniform expression across all cells also underscores the potential of mRNA in therapeutic protein complementation. With uniform expression levels, differences in cell behavior and potential toxicity of compensatory effects for cells showing high expression levels will be less likely.

RIBOPRO PRODUCTS

Off the shelf eGFP mRNA

mRNA properties	
Sequence length	758nt (excluding poly A-tail)
UTRs	RIBOPROs 3' and 5' UTR
5' end	Cap1
Poly A-tail length	150nt

REFERENCES

1. van Asbeck AH, Dieker J, Oude Egberink R, van den Berg L, van der Vlag J, Brock R. Protein Expression Correlates Linearly with mRNA Dose over Up to Five Orders of Magnitude In Vitro and In Vivo. Biomedicines. 2021;9.DOI 10.3390/biomedicines9050511