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Short communication

Transient and stable transfection of *Leishmania* by particle bombardment

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Transfection of trypanosomatids, particularly the transfection of their mitochondrion and different developmental stages, is still a limiting step in many experiments using recombinant DNA technology. Two types of trypanosomatid transfection protocols have been described so far. The standard protocol is based on electroporation first described by Bellofatto and Cross [1]. The second protocol is based on transfection using a homemade particle gun [2]. The biolistic method was first applied to plants [3], but is now routinely used for transfection of a wide variety of targets ranging from bacterial to mammalian cells [4–10] and it is the only method described so far to transfect organelles [10,11].

In trypanosomatids, the genetic organization of the mitochondrion shows several unusual features like a complex DNA network composed of thousands of catenated circular DNA molecules [12], the lack of encoded tRNAs [13] and the still enigmatic process of RNA editing [14]. However, the trypanosomatid mitochondrion is not yet accessible to recombinant DNA technology, and therefore the experimental in vivo analysis of the mentioned features is severely restricted. It is intended that the particle bombardment technique should be applied to transfect mitochondria of trypanosomatids. Since the optimal bombarding conditions for nuclear and organellar transfection of a given organism generally are identical [6,10,15,16] and as a first step, the factors influencing the nuclear transfection efficiency have been investigated.

The ability of microprojectiles to deliver DNA into *Leishmania* cells was demonstrated either by the presence of transient β-glucuronidase (GUS) activity or by selection of stable transformants on plates following bombardment with the plasmid pX63NEO-GUS [17] using the DuPont Biolistic

Abbreviations: GUS, β-glucuronidase.

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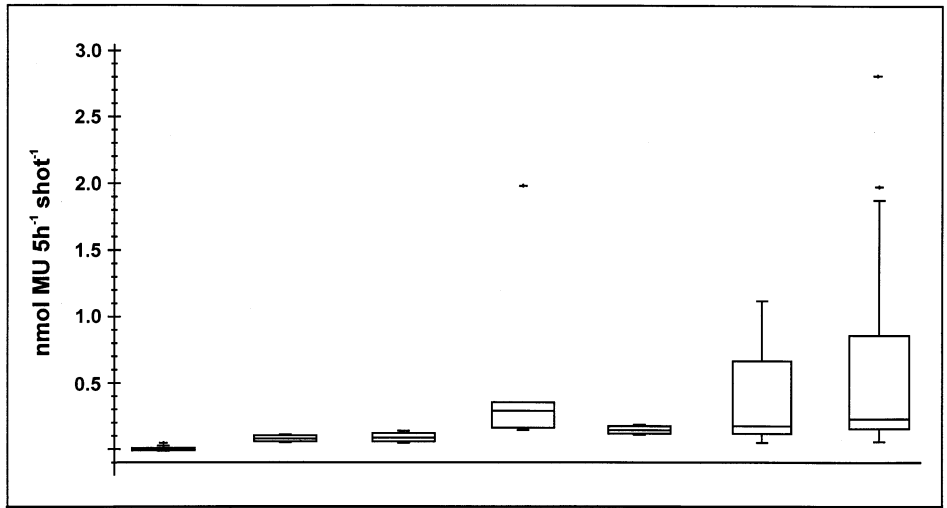


pressure	bombarded without plasmid	1100 ± 200 psi	1400 ± 100 psi	1800 ± 200 psi	1100±200 psi	1400 ± 100 psi	1800 ± 200 psi
pre-treatment		no osmotic pre-treatment			osmotic pre-treatment		
n	0/9	3/12	4/8	6/11	4/4	8/8	19/25

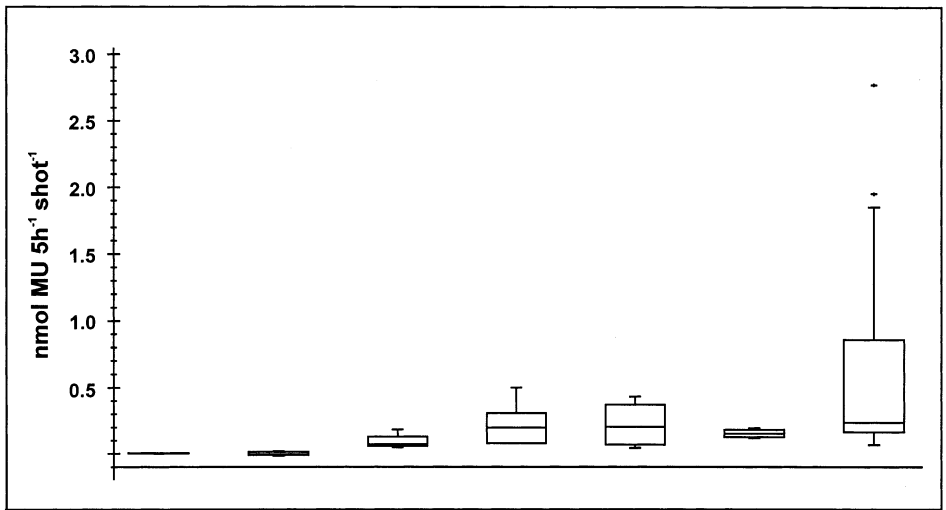
Fig. 1. Optimization of pressure and osmotic pre-treatment. 50×10^6 *L. tarentolae* cells were plated on a pagar [18] consisting of 1% agar solidified BHI +, 3M Whatman paper and a ST68 membrane (Schleicher and Schuell) and were then bombarded with M5 tungsten particles coated with 1 μg of plasmid pX63NEO-GUS using rupture pressure conditions ranging from 900 psi to 2000 psi. To determine the effect of an osmotic pre-treatment, the cells were incubated for 2–3 h with 6% sucrose prior to bombarding. After bombardment, the pagar was overlaid with liquid BHI +. The transfection efficiency was determined measuring the transient reporter gene activity after 42 h. Sample points are summarized in Box-plots showing the 0.1, 0.25, 0.5, 0.75 and the 0.9 percentiles. Extreme values are indicated by crosses. n = number of shots above back-ground compared to the total number of shots.

PDS-1000/He gun. In more than 150 independent bombardments, the effects of different rupture pressures and osmotic pre-treatments (Fig. 1) as well as particle types and sizes (Fig. 2) have been evaluated. The resulting GUS activity was measured fluorometrically [17]. The background fluorescence reached about 3% of the values obtained by optimized bombarding conditions, and no endogenous GUS-activity could be detected. Using tungsten M5 particles, a pressure of 1800 psi and an osmotic pre-treatment with 6% sucrose gave the highest transfection efficiency.

Having established the conditions for transient transfections, a plating assay was applied to determined the efficiency of stable transfection. After bombardment, the cells were cultured overnight in liquid BHI + medium without selective drugs. The cells were then plated on selective plates (1% Agar; 5% FCS, 1:1 mixture of BHI + /M199) containing 100 mg l^{-1} G418. Up to 400 colonies of stable transformants (per μg plasmid) appeared after approximately 2 weeks of incubation at 27°C.

The results showed a high variability, as is characteristic for the biolistic method. The main source of variation has been attributed to particle coating, leading to variable patterns of particle agglomeration [18]. When performing transient transfection assays for the analysis of regulatory sequences, an internal standard, such as a second reporter gene system, could be introduced via co-bombardment leading to highly reproducible results [19,20].

The M5 tungsten particles used here in optimized biolistic transfection experiments are the smallest particles available. They are the most



particles							
size							
type	1μm gold	1μm gold	M10 tungsten	0.6 μm gold	0.6 μm gold	M5 tungsten	M5 tungsten
pressure	1100 ±200 psi	1800 ± 200 psi	1800 ± 200 psi	1100 ± 200 psi	1800 ± 200 psi	1100 ± 200 psi	1800 ± 200 psi
n	0/5	0/5	4/4	5/5	3/4	8/8	19/25

Fig. 2. Optimization of particle type. To determine which particle type gave the best transfection rates, tungsten M5, tungsten M10, and gold particles of 1 and 0.6 μm were tested. The M5 particles are characterized by a diameter of 0.771 μm (average) and a median of 0.362 μm, whereas the M10 have an average diameter of 1.07 μm and a median of 0.64 μm. The highest transfection efficiency was obtained using the M5 particles, all other particles gave significantly lower transfection rates. The use of 1 μm gold particles did not result in activities above the background. All cells were treated with sucrose prior to bombarding as described in Fig. 1. Sample points were collected and summarized as described in Fig. 1.

commonly used particles for transfection of very small biological structures like organelles and bacteria. Furthermore, the bombarding conditions for nuclear and organellar transfection are usually identical. Therefore, it can be concluded that the results presented in this work will be of valuable impact for setting up a mitochondrial transfection system in *Leishmania* in order to study mitochondrial processes such as RNA-editing.

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