Transformation of Nematodes with the Helios™ Gene Gun

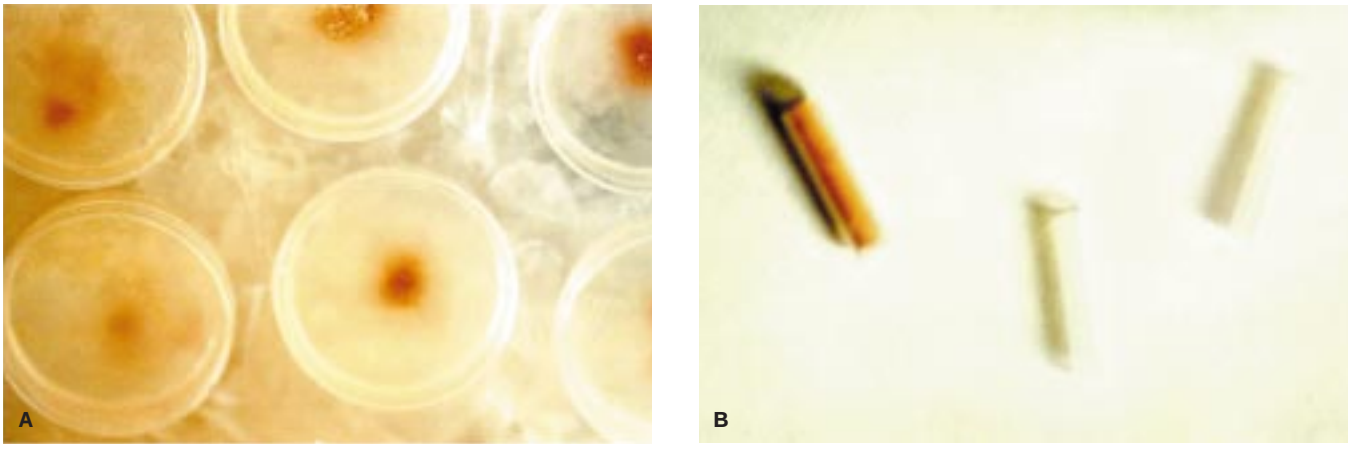

Fig. 1. A. C. elegans *worm pellet in the center of an 6 cm Petri dish on ice after microparticle bombardment (conditions are described in detail in Methods). B. Different Au-DNA containing cartridges (16 mm in length) in comparison to each other. The ¨bullet¨ on the left border, which shows homogeneous distribution of gold-DNA particles, was prepared by our modified protocol.*

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Introduction

The Biolistic® DNA transfer technique was first established for plant cells and plant tissues (Sandford *et al*., 1987; Jefferson *et al*., 1987; Bruce *et al*., 1989; Armaleo *et al.*, 1990). More recent applications have been DNA transfer experiments aiming at DNA vaccination of animals (genetic immunization; Sundaram *et al.*, 1996), for example a bombardment of mice to generate protective antibodies, instead of the DNA injection used for this purpose in the past.

Here we report on the potential to use this convenient method for transfection of nematodes, not only for the free-living soil nematode *Caenorhabditis elegans*, the well known model organism in its class, but also for the cotton rat filaria *Litomosoides sigmodontis*, which serves as model for human pathogenic filariae (Zahner, Hobom and Stirm, 1995), such as *Wuchereria bancrofti* or *Brugia spp*. These parasitic species cause a severe lymphatic disease called filariasis (elephantiasis), from which more than 100 million human beings suffer worldwide.

Methods

PREPARATION OF ADULT HERMAPHRODITE *C. ELEGANS* AND COTTON RAT PARASITE *L. SIGMODONTIS* FOR MICROPARTICLE BOMBARDMENT

C. elegans is cultivated on NGM agar plates (Brenner, 1974). Wild-type worms (strain N2 var. Bristol) are fed on *Escherichia coli* OP-50 (exogenous uracil dependent mutant) seeded on those plates. The surface of a densely populated NGM agar plate is subsequently rinsed with tap water. A drop of water containing nematodes is placed in the center of a 6 cm Petri dish on ice (to reduce their motility). In this state *C. elegans* could be used for microparticle bombardment (Figure 1A).

For the same purpose adult females of *L. sigmodontis* must be isolated from the pleural cavity of their laboratory host *Mastomys coucha* (Wegerhof and Zahner, 1985), and stored in RPMI medium (supplemented with 10% FCS, Gibco-BRL; 1% Penicillin and Streptomycin). Single parasites are transferred to an agar plate and used for microprojectile bombardment.

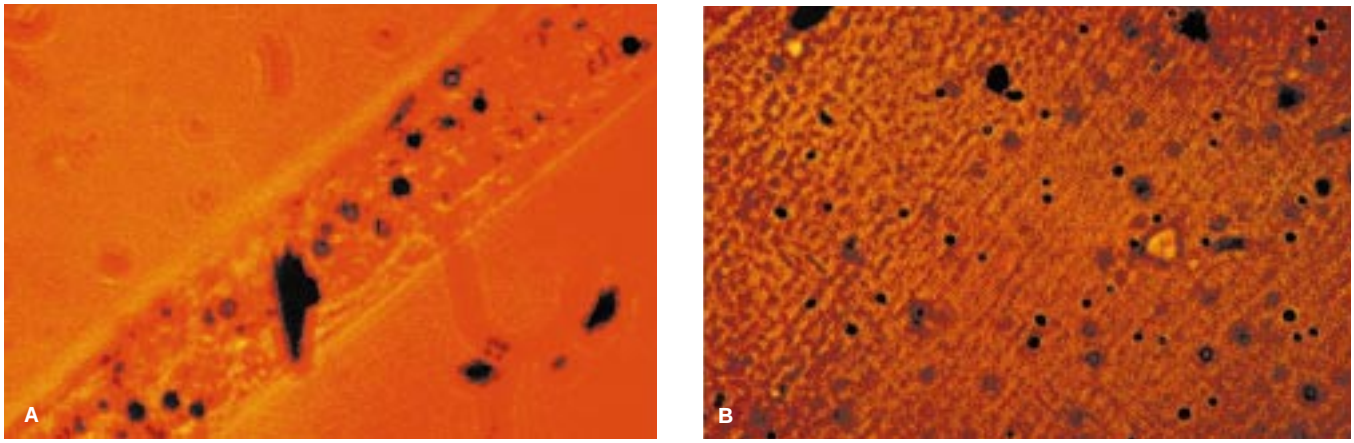


Fig. 2. A. Spherical gold particle located in the body of a living C. elegans *hermaphrodite worm, at 6 hours after bombardment (320x).*
B. Au-particles (diameter 1.0 µ) within the uterus region of an living adult female of L. sigmodontis *(1,000x) directly after bombardment.*

CONDITIONS OF BOMBARDMENT

DNA-coated gold particles are chosen as microparticles following a modified Bio-Rad protocol for the Helios gene gun. The protocol is as follows:

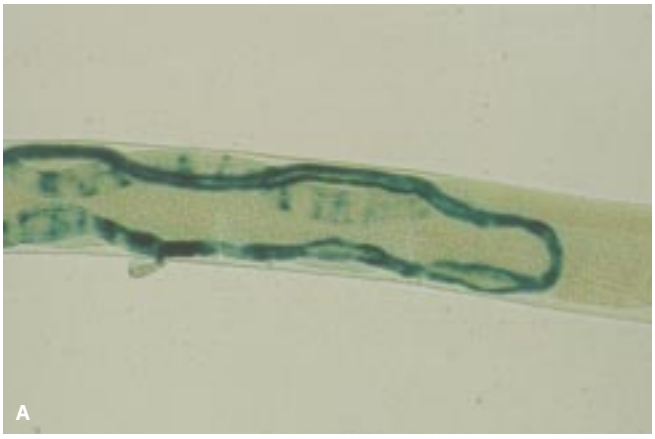
1. Mix 50 mg of gold microparticles (Bio-Rad; a 1:1 mixture of particles 0.6 µ/1.0 µ in diameter) with 100 µl of 0.05 M spermidine (Sigma), and sonicate 3 times for 3 seconds in an Eppendorf® tube (1.5 ml).
2. Add 200 µl of purified supercoiled plasmid DNA to the carrier suspension (in a total volume of 100 µl). It is possible to use more than one plasmid simultaneously.
3. $CaCl_2$ (100 µl, 1 M) is now added dropwise with continuous vortexing. Precipitation was allowed to take place for 10 minutes at room temperature.
4. Wash the suspension three times with 1 ml of absolute ethanol (Sigma-Aldrich), and pellet the sample by centrifugation (12,000 rpm for 1 minute, Heraeus Biofuge).
5. Finally, the pellet is resuspended in 3.5 ml of ethanol containing 0.1 mg/ml polyvinylpyrrolidone (PVP; MW 360,000). In this state a DNA-coated gold particle suspension can be stored for up to 2 months at -20 °C (if not directly used for cartridge preparation).

USING THE TUBING PREP STATION

1. Clean the inner surface of the Tefzel tubing (external size 10 mm; internal diameter 6 mm; Bio-Rad) with 5 ml of absolute ethanol and dry with nitrogen (grade 4.8; 99.998% pure) in the rotating tubing holder for at least 5 minutes.
2. Sonicate the DNA-coated particle suspension in ethanol for 3 seconds (to achieve a homogeneous solution) and load it into the tubing at a rate of 5.5–6 ml/min using a syringe.
3. Allow the DNA-coated particles to settle for 3 minutes.
4. Remove the ethanol solution quickly (within 30 seconds).
5. For drying the wall-attached particles, flush nitrogen gas through the tubing for 3 minutes at a flow rate of 0.35 l/min.
6. Finally, cut the resulting particle-covered tubing into individual cartridges 16 mm in length (one full length of tubing results in nearly 50 cartridges) to fit into the 12-shot barrels of the Helios gene gun (Bio-Rad). See Figure 2.
7. In a desiccated atmosphere, these can be stored at 4 °C for up to 8 months without losing activity.
8. Deliver the DNA-coated gold particles using 4 blasts of helium (grade 4.5; 99.995% pure) applied to the cartridges at a pressure of 300 p.s.i. and a distance of 3 cm to the target (in the case of *C. elegans*).
9. A pressure of 120–180 p.s.i. at a distance of 2 cm using 1 blast of helium is useful for the transfer into parasitic nematode *L. sigmodontis*. After this procedure, the female parasites may be cultivated at 37 °C for up to 2 weeks in RPMI medium (5% CO_2).

SELECTION FOR TRANSFORMED TRAITS OF *C. ELEGANS*

While the majority (up to 90%) of *C. elegans* hermaphrodites are killed during Biolistic DNA transfer, the survivors with their gold particles incorporated may be cultivated on new *E. coli*-seeded NGM agar plates (Figure 2A shows particles within a living hermaphrodite). F1/F2 progeny nematodes were screened 3 to 4 days later for visible dominant mutant marker rol-6 (Kramer *et al*., 1990). Animals carrying roller allele su-1006 exhibit a helically twisted cuticle and body. Muscle contractions, which would normally propel the worm forward or backward in a sweeping sinusoidal motion, will cause the nematode instead to roll over its longitudinal axis and to move in circles (Mello *et al*., 1991; Fire, 1986; Kramer *et al*., 1990). In the case of *L. sigmodontis*, no special selection mode is necessary because the success of bombardment can be visualized by standard light microscopy to make visible the gold particles now located within these filariae (see Figure 2B).



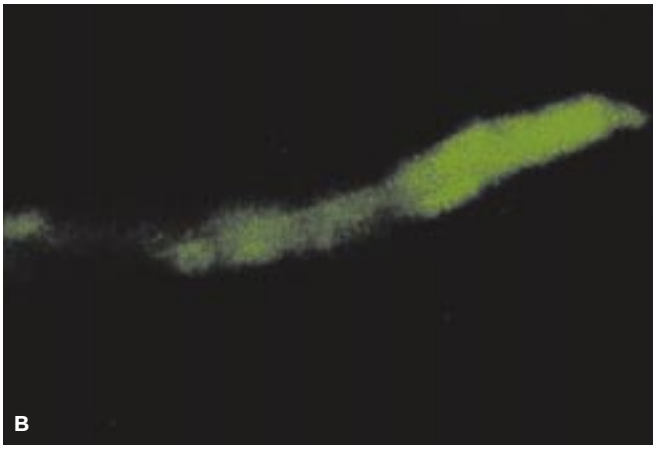


Fig. 3. A. Histochemical analysis of E. coli *ß-galactosidase activity under transcriptional control of the SV 40 early promoter in 77 days old adult female* Litomosoides sigmodontis *two days after bombardment with DNA-coated gold particles (1.0 µ). A surviving, active animal was kept for 48 hour in 10% FCS and 1% antibiotic containing medium at 37 °C (pCO_2:5%), and upon acetone treatment was used for staining with standard ß-galactosidase substrate X-gal. The picture demonstrates (at 60x magnification) the blue indigo reaction product exclusively in the uterus wall and ovarian tissues of the nematode used for DNA transfer, while other parts of the same animal's body do not show ß-galactosidase expression. B. Adult hermaphrodite of* C. elgans *(F4 generation) after initial bombardment showing ß-galactosidase activity (320x) under the control of SV 40 early promoter. Staining procedure was as described above.*

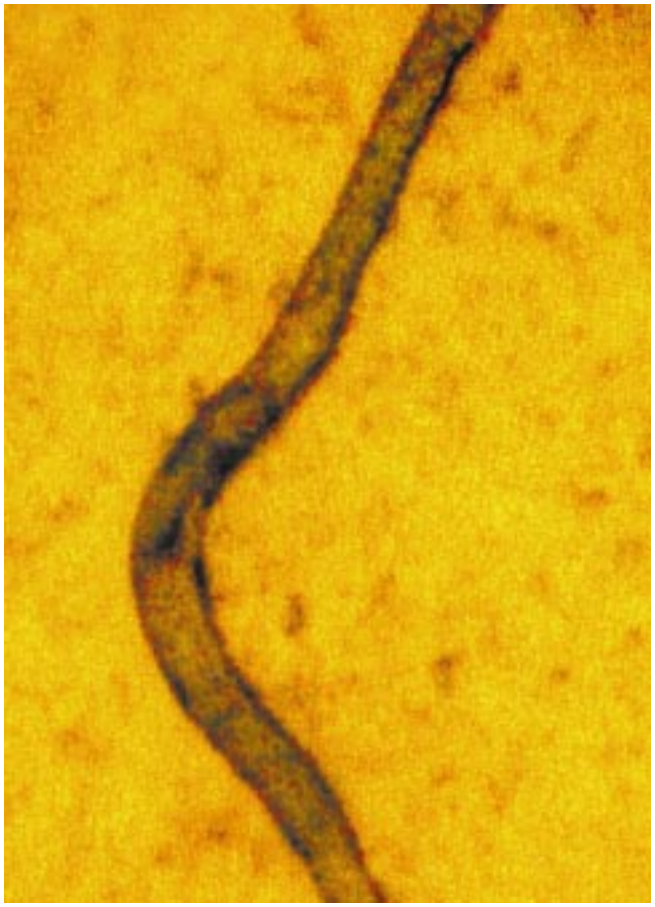


Fig. 4. Ubiquitous expression of mutant GFP (with enhanced fluorescence) under the transcriptional control of the C.elegans *actin-1 core promoter in F3/F4 adult hermaphrodite (250x) after to the DNA transfer step. GFP expression was recorded using a Zeiss Axioplan fluorescence microscope (excitation BP 470 (+/-20) nm; emission BP 505–530 nm, beamsplitter FT493, filterset 13/488013 GFP), and documented like all pictures on Kodak 400 ASA diafilm.*

Results

The established mode of DNA transfer into *C. elegans* is microinjection of DNA into the gonadal syncytium (Stinchcomb *et al*., 1985; Fire, 1986). Under standard selection conditions promoter-reporter gene cDNA constructs are used, in co-transfection with a visible dominant such as mutation rol-6 (su1006), which leads to roller movement phenotype of transformed worms (already in F1 generation) and is easily detectable within a background of normally moving animals. The same transfection efficiency has been observed for this Biolistic DNA transfer method as for microinjection experiments (Fire, 1986), while this new strategy is less time consuming and much more convenient for co-transfection experiments. In the F1 generation, DNA transfer rates of up to 1% were observed using this selection method, which became considerably enriched during following generations after further transfer (F3/F4: 20%; F5/F6 up to 90%), proving their genetic fixation.

Besides marker gene rol-6, we have employed two different reporter genes, a mutant form of GFP (green fluorescent protein), which was originally cloned from the jellyfish *Aequorea victoria* (Prasher *et al*., 1992; Cody *et al*., 1993; Chalfie *et al*., 1994) with enhanced fluorescence (Siemering *et al*., 1996), and ß-galactosidase from *Escherichia coli*. Expression was visualized by standard fluorescence microscopy (GFP; see Figure 4) or by histochemical staining (lacZ).

In both cases we have observed marker gene expression under transcriptional control of the SV40 early promoter (Figure 3 A and B), or of the *C. elegans* actin-1 core promoter after Helios gene gun mediated delivery of Au/DNA particles into the nematode. The lacZ expression of constructs containing the actin-1 core promoter of *C. elegans* (Files *et al*., 1983; Krause *et al*., 1989; Stone and Shaw, 1993) in DNA transfected *L. sigmodontis* will even be analyzed histochemically.

Discussion

The Biolistic DNA-transfection procedure, because of its simple mode of handling, has an important potential in various innovative molecular biology applications, such as DNA vaccination, protein targeting, and basic research applications such as gene regulation studies. The new Biolistic technology should therefore be able to substitute for other applications in this field. Also, the prepared DNA/Au particles can be stored for rather long periods, at least up to 8 months, which makes repetitions or comparative experiments more meaningful. In applying this technique to various nematodes, it is conveniently possible to use a couple of hundred target worms of *C. elegans* and proceed in comparatively low precision, while DNA injections have to be done individually and precisely into the gonads. Parasitic worms, however, can only be transfected on an individual basis or in small groups by Biolistic gene transfer. In all experiments designed, reporter gene expression is observed independent from distance of initial gold particle target sites, clearly indicating an DNA drift within the entire bodies. Altogether, this technique is a reproducible and efficient method of producing fertile transgenic nematodes.

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