Transient expression of DNA after ballistic introduction into *Drosophila* embryos

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Current procedures for introduction of DNA into *Drosophila* embryos require the time-consuming microinjection of DNA solution into individual embryos (1, 2). A procedure has recently been used successfully in plants, yeast and cultured cells for the ballistic introduction of DNA via tungsten (W) particles (3–5). Using a modification of this procedure for *Drosophila* embryos, we have obtained transient expression at high efficiency. The technique also has the potential to be useful for germ line transformation.

All incubations and procedures were at room temperature. DNA [a total of 1–20 μ g of a 1 μ g/ μ l solution in embryo injection buffer (0.1 mM sodium phosphate pH 6.8, 5 mM KCl)] was precipitated onto washed W particles (1.2 μ m diameter, obtained from Biolistics, Inc.) in a total volume of 65 μ l, according to the recommendations of the manufacturer. After the DNA precipitation step, 50 μ l of supernatant was removed (without centrifugation). The tube containing the remaining pellet and residual supernatant (12–15 μ l) was vortexed just before applying 8 μ l of this sample to the macroprojectile. Embryos (approximately 10–20,000), collected for 1 hr from a cage of *w*¹¹¹⁸ flies onto yeasted apple juice agar plates, were dechorionated for 3 min with 50% bleach and spread out on a circle of wet Whatman #1 filter paper. The filter was then blotted on paper towels, transferred to a fresh apple juice agar plate (10 $\times$ 1.5 cm) and the embryos on the filter bombarded with the DNA-carrying W particles, using a Biolistics bioparticle delivery system (DuPont) (3).

Transient expression was assessed by bombarding with particles carrying DNA of the P160 construct, a plasmid containing the actin 5C promoter fused to the *E. coli* β -galactosidase gene (J.-L. Couderc, personal communication). The plate of bombarded embryos was incubated overnight, then fixed and stained with X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) using standard procedures (6). The majority of the embryos survived and were stained blue; many were very dark, indicating strong expression of β -galactosidase (Figure 1). Testing of several variables in the procedure revealed that dechorionation greatly increased the number of blue-stained embryos, that Whatman filter paper was superior to Nitex as a backing to support the embryos during the blasting, and the number of shots per plate did not significantly increase the number of stained embryos. Increasing the DNA per bombardment from 1 to 20 μ g increased the number of stained embryos.

Figure 1. Transient β -galactosidase expression in W particle bombarded *Drosophila* embryos. Embryos were bombarded with 10 μ g of plasmid P160 DNA, incubated 20 hrs, fixed and stained with X-gal. Embryos shown are from an area near the center of the plate. Note the high proportion of stained embryos, as well as embryos with intense staining.

Potential for germ line transformation. We attempted germ line transformation using a mixture of the helper plasmid, p π 25.7wc (7), and the P-element vector pCasPeR (8) in a 1:1.7 ratio. From a bombardment with a total of 100 μ g of DNA, one *w*⁺ B1 fly was obtained in the progeny generation. This transformant had light orange eyes, typical of transformants obtained with the pCasPeR vector, and was used to generate a line with light orange eye color. Southern blot hybridization to genomic DNA prepared from this line was consistent with it containing pCasPeR DNA.

Our results show that ballistic methods are suitable for the introduction and transient expression of DNA into *Drosophila* embryos, and thus the rapid evaluation of β -galactosidase fusion constructs. The technique could probably be used to study the quantitative effects of transcription regulatory elements by assaying homogenates of bombarded embryos for both concentration of the ballistically introduced construct DNA and for an enzyme expressed transiently from it. Although only one germline transformant was obtained, this result is very encouraging, given the simplicity of the technique. Further modifications, such as the addition of sequences to localize DNA to the posterior, and the bombardment of embryos which carry the Δ 2–3 construct expressing the P element transposase (9), might improve the transformation rate to a level which would make bioparticle bombardment a viable alternative to microinjection.

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