

frequencies of 1–6 kHz and 12–25 kHz, thresholds between cells with distinct characteristic frequencies differ by 20–30 dB at a given frequency (Fig. 1b), thus providing a unique facility (for insects) for frequency discrimination in the auditory pathway.

Given the uniform design of the auditory system in cicadas⁷, it is possible that the frequency-modulated songs of many cicada species, particularly tropical ones⁹, may result from sensory drive¹⁰, because females are able to use frequency components of songs as criteria for species recognition and mate choice.

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1. Bradbury, J. W. & Vehrencamp, S. L. *Principles of Animal Communication* (Sinauer, Sunderland, MA, 1998).
2. Hoy, R. R., Popper, A. N. & Fay, R. R. (eds) *Comparative Hearing: Insects* (Springer, Berlin, 1998).
3. Römer, H. *Nature* **306**, 60–62 (1983).
4. Oldfield, B. P. *Trends Neurosci.* **11**, 267–270 (1988).
5. Römer, H. *Phil. Trans. R. Soc. Lond. B* **226**, 179–185 (1993).
6. Stumpner, A. J. *Neurophysiol.* **79**, 2408–2415 (1998).
7. Fonseca, P. J. *Acoustic Communication in Cicadas (Homoptera, Cicadoidea): Sound Production and Sound Reception*. Thesis, Univ. Lisbon (1994).
8. Popov, A. V. in *Sensory Systems and Communication in Arthropods* (eds Gribakin, F. G., Wiese, K. & Popov, A. V.) 301–304 (Birkhäuser, Basel, 1990).
9. Gogala, M. *Bioacoustics* **6**, 101–116 (1995).
10. Endler, J. A. *Am. Nat.* **139**, 125–153 (1992).

Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

Biotechniques

Transfection of cells by immunoporation

Cell transfection is now a central technique in molecular biology and an essential prerequisite for gene therapy. Here we describe how beads coated with antibodies and bound to specific cell-surface transmembrane proteins can create holes in cells when the beads are removed, allowing transfection of the cells with DNA or other macromolecules. This unique targeted transfection of cells by immunoporation is very efficient and results in minimal cell death.

A variety of methods have been developed for the transfection of cells, including electroporation^{1,2}, lipofection^{3,4}, calcium phosphate coprecipitation^{5,6} and DEAE dextran^{7,8}. Of these methods, only electroporation offers the possibility of introducing DNA and other molecules such as proteins into viable cells. None of the current methods is able to target specific types of cells for transfection. In this new method

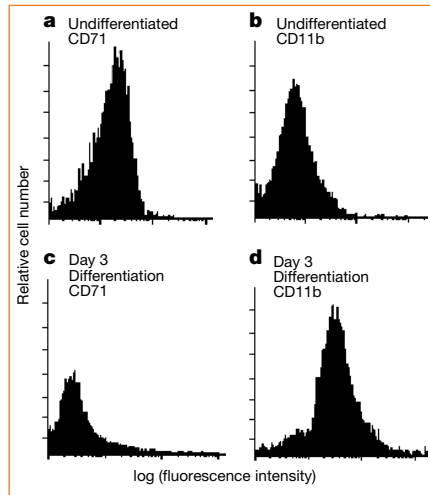


Figure 1 Analysis of the transfection of HL-60 cells with pEGFP-C1 by measurement of the expression of green fluorescent protein using flow cytometry. **a**, HL-60 cells transfected using DYNAFECT-CD71 beads; **b**, HL-60 cells transfected using DYNAFECT-CD11b beads; **c**, DMSO-induced HL-60 cells transfected using DYNAFECT-CD71 beads; **d**, DMSO-induced HL-60 cells transfected using DYNAFECT-CD11b beads.

of cell transfection, antibody-coated beads are bound to specific surface antigens and then the beads are sheared off from the cell by mixing: this causes the formation of transient holes in the cell membrane through which macromolecules can enter.

Granulocytic, differentiating human lymphoblastic HL-60 cells normally express CD71 on their surfaces. When induced to differentiate in the presence of dimethyl sulphoxide (DMSO), the cells cease to express CD71 and instead express CD11b. We have used this cell line as a model system to investigate the process of cell transfection mediated by immunoporation.

DYNAFECT beads coated with either anti-CD11b antibody (DYNAFECT-CD11b) or anti-CD71 antibody (DYNAFECT-CD71) were mixed on a rotating end-over-end mixer at 33 r.p.m. for 6 h at 22 °C with either uninduced HL-60 cells or cells that had been induced with DMSO for 3 days. For mixing in a 2-ml microcentrifuge tube, 10⁷ beads and 5 × 10⁵ cells were suspended in 0.5 ml transfection medium (Dyna AS) containing 0.2 µg plasmid DNA vector pEGFP-C1 (4.7 kilobases) which codes for green fluorescent protein. After transfection, the beads were removed using a magnetic separator and the cells were transferred back into tissue-culture medium and cultured for a further 48 hours before analysis.

The extent of cell transfection was determined by flow cytometry. Figure 1 shows that the DYNAFECT-CD71 beads facilitate the transfection of DNA into normal HL-60 cells, but when the cells become differentiated and no longer express CD71, transfection no longer occurs with these beads. In contrast, mixing undifferentiated HL-60

cells with DYNAFECT-CD11b beads does not result in the transfection of the cells with DNA, but when the cells are differentiated and begin to express CD11b, those beads do bring about transfection.

Hence, immunoporation has the potential to target specific types of cell in a mixed population for transfection, depending on their immunological identity, and allow the targeted cells to take up a variety of different molecules. This also occurs in several mammalian cell lines with a range of different antibodies that target selected cell-surface antigens. In all cases, the level of transfection was 40–80%, depending on mixing conditions, and non-viable cells usually numbered less than 20%.

The high levels of selectivity and transfection, together with minimal cell death, that are achievable with immunoporation illustrate the enormous potential of this technique for use in a wide range of transfection studies. In particular, the ability to target specific subpopulations of cells will be extremely useful for many gene therapy applications.

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1. Fromm, M., Callis, J., Taylor, L. P. & Walbot, V. *Methods Enzymol.* **153**, 351 (1987).
2. Pfau, J. & Youderian, P. *Nucleic Acids Res.* **18**, 6165 (1990).
3. Watanabe, Y. *et al. J. Biochem.* **116**, 1220–1226 (1994).
4. Hargest, R., Eldin, A. & Williamson, R. *Adv. Exp. Med. Biol.* **451**, 385–391 (1998).
5. Ambrosini, E. *et al. J. Neurosci. Res.* **55**, 569–577 (1999).
6. Nielson, T. *et al. Mol. Gen. Genet.* **242**, 280–288 (1994).
7. Muck, K. D., Wei, R. & Elbagarri, A. *J. Immunol. Methods* **211**, 79–86 (1998).
8. Yang, Y. W. & Yang, J. C. *Biotechnol. Appl. Biochem.* **25**, 47–51 (1997).

Erratum

Non-haemolytic β-amino-acid oligomers

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Some symbols representing haemolytic activity were absent or incorrect in Fig. 1c. The correct figure is shown here. Crosses, β-17; circles, magainin derivative; squares, melittin.

