REVIEW ARTICLE

Microinjection of somatic cells with micropipettes: comparison with other transfer techniques

Julio E. CELIS

Division of Biostructural Chemistry, Department of Chemistry, Aarhus University, Langelandsgade 140, DK-8000 Aarhus C, Denmark

Introduction

A handful of techniques to introduce macromolecules into somatic cells have been developed during the last decade (for references see Poste *et al.*, 1976; Graham, 1977; Schlegel & Rechsteiner, 1978; Graessmann *et al.*, 1979*b*, 1980*a*; Kulka & Loyter, 1979; Celis *et al.*, 1980*a*; Baserga *et al.*, 1980; Gregoriadis & Allison, 1980; Furusawa, 1980; Paraf & Nicolau, 1981; Gregoriadis *et al.*, 1982; Rechsteiner, 1982; Nicolau *et al.*, 1983*a*; Wu *et al.*, 1983; Evered & Whelan, 1984). These techniques, which aim at using the somatic cell as a living test tube, can be divided in three types depending on the way the macromolecules are delivered to the recipient cells.

(1) Direct microinjection

Microinjection with micropipettes (Graessmann, 1968; Graessmann & Graessmann, 1971; Diacumakos, 1973; Stacey & Allfrey, 1976; Celis, 1977; Capecchi, 1980).

(2) Vehicle-mediated transfer

Erythrocyte ghosts (Furusawa *et al.*, 1974, 1976; Loyter *et al.*, 1975; Schlegel & Rechsteiner, 1975; Kaltoft & Celis, 1978; Krieger *et al.*, 1978; Schlegel & Mercer, 1980; Uchida *et al.*, 1980; Rechsteiner, 1982).

Liposomes (Gregoriadis & Buckland, 1973; Papahadjopoulos *et al.*, 1974*a,b*, 1980*a,b*; Pagano *et al.*, 1974; Poste *et al.*, 1976; Pagano & Weinstein, 1978; Fraley *et al.*, 1979, 1980; Poste, 1980; Gregoriadis, 1980; Wong *et al.*, 1980; Nicolau & Sene, 1982; Nicolau *et al.*, 1983*a*).

Reconstituted Sendai virus envelopes (Uchida *et al.*, 1977; Volsky *et al.*, 1979; Loyter & Volsky, 1979, 1982; Vainstain *et al.*, 1983; Beigel & Loyter, 1983; Loyter *et al.*, 1983, 1984).

(3) Transfer aided by facilitators

Calcium phosphate (Graham & van der Eb, 1973; Wigler *et al.*, 1978; Graham *et al.*, 1979; Lewis *et al.*, 1980).

DEAE-dextran (Vaheri & Pagano, 1965; Pagano & Vaheri, 1965; McCutchan & Pagano, 1968; Warden & Thorne, 1968; Graham, 1977).

Poly(ethylene glycol)/sucrose shock (Chu & Sharpe, 1981; Shen *et al.*, 1982).

Osmotic lysis of pinosomes (Okada & Rechsteiner, 1982).

The first three techniques have been used mainly for DNA-mediated gene transfer, while osmotic lysis of pinosomes has been used to introduce proteins (Okada & Rechsteiner, 1982).

To date, a wide range of macromolecules (DNA, drugs, lipids, proteins, RNA, small molecules) as well as organelles (chromosomes, nuclei, membrane fragments) and virus particles have been introduced or transplanted into somatic cells by using these techniques. It is now clear, however, that there are limitations inherent in each one of these techniques and that certain biological problems are best approached using a particular technique. The purpose of this short Review is to evaluate the present stage of the technology of direct microinjection of somatic cells with micropipettes and to emphasize the areas of research in which this technique has uniquely contributed to our advance in knowledge. A description and evaluation of the other transfer techniques is given for comparison.

Direct microinjection

Microinjection of somatic cells with micropipettes

The technique of microinjection of somatic cells with micropipettes was developed originally by Graessmann (1968) and Diacumakos (1973). The technique is based on the use of small micropipettes (0.5–1 μm diameter at the tip) which allow the direct transfer of macromolecules into recipient somatic cells grown on a solid support or artificially bound to a substrate (Graessmann *et al.*, 1980*c*; see also the legend to Fig. 1). This technique has no cell-type restriction and the macromolecules can be delivered either into the cytoplasm (Figs. 1*a* and 2) or the nucleus (Fig. 1*b*) of the recipient cells.

Fig. 1. Micrograph from a television monitor showing cytoplasmic (a) and nuclear (b) injections (from Celis *et al.*, 1980b)

The glass coverslip (144 or 9 mm²) with the attached cells is placed in a 5 cm Petri dish containing 8 ml of warm medium minus serum or Hank's buffered solution. The cells are viewed under phase contrast ($\times 300$). Once the cells are focused the tip of the micropipette is lowered until it is in the same focal plane as the cells. To inject the cell, we position the tip of the micropipette so that it lies just outside the cell. It is necessary just to touch the cells in order to penetrate them. The pipette is connected to a 50 ml glass syringe, to assist the injection of liquid. Injection is assessed visually. Once a cell has been injected, the micropipette is raised and moved closer to the next cell to be injected. The procedure is simple and about 100 cells can be injected in 15 min. Various microinjection set-ups are currently available and these are described in articles by Graessmann (1968), Diacumakos (1973), Celis (1977), Yamamoto & Furusawa (1978), Stacey (1981), Ansoorge (1982), Graessmann & Graessmann (1983a) and Timm *et al.* (1983).

To assess the possibilities of the direct microinjection and to allow a comparison with other transfer methods (see also Table 1), it is necessary to assess the advantages and disadvantages of this technique. The main advantages can be summarized as follows.

(1) A wide variety of recipient cultured somatic cells (primary or secondary cultures) can be used. Even cells that grow in suspension can be microinjected if bound to a substrate by a suitable linker

[concanavalin A, poly(L-lysine) or phytohaemagglutinin; Graessmann *et al.*, 1980c].

(2) Only a small amount of sample is required; usually about 1–2 μ l is sufficient.

(3) The average number of molecules injected per cell can be estimated. About 5×10^{-11} – 10^{-10} ml can be injected per cell without any significant effect on cell viability (Stacey & Allfrey, 1976; Celis, 1977; Graessmann & Graessmann, 1983a,b).

(4) There is no apparent limitation on the size or type of macromolecules that can be injected. To date, DNA (for references see Graessmann *et al.*, 1976, 1978, 1979b, 1981; Mueller *et al.*, 1978; Capecchi, 1980; Anderson *et al.*, 1980; Kopchick *et al.*, 1981; Kopchick & Stacey, 1984; Timm *et al.*, 1983; Pugatsch & Stacey, 1982; Graessmann & Graessman, 1983a,b, 1984), RNA (for references see Graessmann *et al.*, 1974, 1976, 1982; Graessmann *et al.*, 1974; Stacey & Allfrey, 1976; Celis, 1977, 1984; Stacey *et al.*, 1977; Celis *et al.*, 1980b,c; Stacey, 1980a,b), proteins (for references see Stacey & Allfrey, 1977; Tjian *et al.*, 1978; Feramisco, 1979; Kreis *et al.*, 1979; Wehland & Weber, 1980; Klimowsky, 1981; Lin & Feramisco, 1981; Mercer *et al.*, 1983; Glacy, 1983), small molecules, organelles and virus particles have been introduced into the cytoplasm or the nucleus of a wide range of cultured somatic cells. DNA species are efficiently expressed following microinjection (Table 1; nearly 100% for transient expression) and stable TK⁺ transformants have been obtained with frequencies of 0.1–0.2% of the intranuclearly injected cells (Table 1; Capecchi, 1980). mRNA species (Table 1; for references see Celis, 1984) and complementary RNAs (Graessmann & Graessmann, 1976, 1982) are readily translated and suppressor tRNA species have been shown to correct nonsense mutations (Hudziak *et al.*, 1982). Microinjected proteins have been shown to find their cellular localization (Fig. 2; microinjection of T-antigen) and to exhibit biological activity. Often, however, injected proteins do not find their cellular localization, a fact that is worrisome.

(5) The biological effect of the injected molecules can be analysed in a natural environment. Assays can be performed in single cells if autoradiographic, immunofluorescent or histochemical tests are available. At the protein synthesis level the effect can be assessed in 10–50 injected cells if improved [³⁵S]methionine labelling techniques (Bravo *et al.*, 1981; Celis & Bravo, 1981) are used in combination with polyacrylamide-gel electrophoresis (Bravo & Celis, 1980; Celis *et al.*, 1980b; Graessmann & Graessman, 1982).

(6) The injected cells can be followed after injection.

Table 1. Comparison between the various transfer techniques

Technique	DNA					
	Thymidine kinase gene		SV40 genes		Proteins	
	Cells expressing thymidine kinase (%)	TK ⁺ colonies (%)	Number of molecules of SV40 DNA required for plaque formation	Cells expressing T-antigen (%)	(average number of molecules injected per cell)	mRNA (average number of molecules injected per cell)
Direct microinjection						
Microinjection with micropipettes	50-100 ^a	0.1-0.2 ^b	1 ^f	100 ^g	Serum albumin 2.4 × 10 ^{7j}	Protamine mRNA (3-6) × 10 ^{5m} 2.9 × 10 ^{4o}
Vehicle-mediated transfer						
Erythrocyte ghosts		0.005-0.0017 ^c				
Liposomes	10 ^a	0.02 ^a	10 ^{6f}		2.7 × 10 ^{6k}	(1.2-2.4) × 10 ⁴ⁿ
Reconstituted Sendai virus envelopes		0.08-0.10 ^d	5 × 10 ^{5f}	1-20 ^h	(1-4) × 10 ^{4l}	1 × 10 ^{4p}
Transfer aided by facilitators						
Calcium phosphate	3 ^a	0.05 ^a	10 ^{6f}	5-10 ⁱ		
DEAE-dextran	0.1-1 ^a	<0.0001 ^a				
Poly(ethylene glycol)/sucrose shock		≈0.03 ^e		70 ^e		
^a From Schäfer-Ridder <i>et al.</i> (1982).						
^b Capecchi (1980).						
^c Iino <i>et al.</i> (1983). Correspond to the highest value reported.						
^d Vainstain <i>et al.</i> (1983). Correspond to the highest value reported.						
^e Shen <i>et al.</i> (1982). Correspond to the highest value reported.						
^f From Graessmann & Graessmann (1984).						
^g Graessmann <i>et al.</i> (1976).						
^h Loyter <i>et al.</i> (1983).						
ⁱ Rassoulzadegan <i>et al.</i> (1982).						
^j These data have been calculated assuming injection of 10 ⁻⁷ µl of a 1.7 mg/ml solution of bovine serum albumin.						
^k Wassermann <i>et al.</i> (1976).						
^l Cited in Loyter & Volsky (1982).						
^m These data have been calculated assuming injection of 10 ⁻⁷ µl of a 1 mg/ml solution of protamine mRNA.						
ⁿ Boogard & Dixon (1983b).						
^o These data have been calculated assuming injection of 10 ⁻⁷ µl of a 0.1 mg/ml solution of globin mRNA.						
^p Ostro <i>et al.</i> (1980).

Fig. 2. *Intranuclear T-antigen accumulation*
Purified D2 T-antigen molecules were microinjected into the cytoplasm of recipient cells. Cells were fixed and stained with anti-T serum at various times (a, immediately; b, 30 min; c, 2 h) after microinjection. From Graessmann *et al.* (1980b).

The main disadvantages of the technique can be summarized as follows.

(1) The number of cells that can be injected is limited. An experienced researcher can inject a maximum of about 1000 cells/h. Thus, the technique cannot be used to study biological or biochemical processes that require a much higher number of cells. This disadvantage can be partially overcome by microinjecting multinucleated giant cells produced by poly(ethylene glycol)-induced fusion of cultured cells (Graessmann *et al.*, 1979a). These cells, however, are not viable.

(2) The technique cannot be used to deliver macromolecules into cells of living organisms or to implant components into the membrane of cultured somatic cells.

(3) There is a damaging effect produced by the introduction of the micropipette and by the pressure exerted by the injected fluid. The extent of the damage cannot be estimated at present.

The main areas of research in which this technique has uniquely contributed to an advance in

knowledge are: cell transformation, regulation of gene expression, gene transfer in cultured cells, studies of the cytoskeleton, translation of mRNA species, etc.

Vehicle-mediated transfer

Erythrocyte-ghost-mediated transfer

The erythrocyte-ghost-mediated transfer is based on the fusion of red blood cells, preloaded with macromolecules, together with somatic cells by using inactivated Sendai virus (Furusawa *et al.*, 1974; Loyter *et al.*, 1975; Schlegel & Rechsteiner, 1975) or poly(ethylene glycol) (Kaltoft & Celis, 1978; Krieger *et al.*, 1978) as fusogenic agents. First, the macromolecules are trapped within the erythrocyte ghosts during hypo-osmotic haemolysis (step 1; Fig. 3) and secondly the resealed vesicles are fused with the recipient cells (step 2; Fig. 3) (see also Schlegel & Rechsteiner, 1978; Rechsteiner, 1982). The average number of ghosts fusing with recipient cells is about 5 in poly(ethylene glycol)-induced fusions as compared with 1–2 for Sendai virus fusions. More recently, Schlegel & Mercer (1980) have improved the efficiency of microinjection by combining the use of poly(ethylene glycol) (fusogenic agent; 44%) and the lectin phytohaemagglutinin (50–75 µg/ml). Under these conditions 70–75% of the quiescent fibroblasts could be injected with a viability of 80–95%. Protocols have also been developed for antibody-directed targeting of ghosts to cell surface antigens (Godfrey *et al.*, 1981, 1983). For example, avidin-coupled erythrocytes have been successfully fused to mouse lymphocytes labelled with biotin-anti-(mouse immunoglobulin) (Godfrey *et al.*, 1983).

By this technique, macromolecules are introduced into the cytoplasm of the recipient cultured cells. For those macromolecules that do not usually cross the nuclear membrane, access to the nucleus can be achieved by using cells in mitosis (R. A. Schlegel, S. L. Miller & K. M. Rose, unpublished work). Proteins (for references see Schlegel & Rechsteiner, 1975, 1978; Loyter *et al.*, 1975; Wassermann *et al.*, 1976; Rechsteiner & Kuehl, 1979; Zavortink *et al.*, 1979; Yamaizumi *et al.*, 1979; Antman & Livingston, 1980; Bigelow *et al.*, 1981; Chin *et al.*, 1982; Rechsteiner, 1982; McGarry *et al.*, 1983; Rechsteiner *et al.*, 1984; R. A. Schlegel, S. L. Miller & K. M. Rose, unpublished work) and RNA (Kaltoft *et al.*, 1976; Capecchi *et al.*, 1977; Anderson *et al.*, 1977; Boogard & Dixon, 1983a,b) have been transferred into somatic cells by using this technique but the average number of molecules injected per cell is about 10 times lower than that achieved by the direct microinjection (Table 1; calculations are based on the injection of bovine serum albumin).

Fig. 3. Schematic representation of the erythrocyte-ghost-mediated transfer

(1) Erythrocyte loading, (2) fusion of loaded ghosts with recipient cells, and (3) transfer of macromolecules to the recipient cells. (a)–(c) Illustrate the above steps by using fluorescein isothiocyanate-conjugated bovine serum albumin. Modified from Celis (1977) and Kaltoft & Celis (1978).

There are a few reports concerning transfer of DNA (Iino *et al.*, 1983; Wiberg *et al.*, 1983), but this requires special loading conditions. For example, in the protocol described by Wiberg *et al.* (1983) both the presence of EDTA and repeated freeze–thawings of the ghosts during loading are required to trap DNA. The reported efficiency of transformation (TK^+ transformants) is, however, significantly lower than that achieved by the direct microinjection approach, by the reconstituted Sendai virus envelope method or by the calcium phosphate technique (Table 1).

The main advantage of this technique over the direct microinjection is that a large number of cells (10^7 – 10^8) can be injected simultaneously. The main disadvantages are the co-transfer of residual erythrocyte components and the need for a fusogenic agent. This technique has uniquely contributed to studies of degradation and turnover of proteins (for references see Neff *et al.*, 1981; Katznelson & Kulka, 1983; Rechsteiner *et al.*, 1984).

Liposome-mediated transfer

In this technique, the macromolecules are first trapped within phospholipid vesicles (liposomes) before delivery to the recipient cells (Gregoriadis & Buckland, 1973; Papahadjopoulos *et al.*, 1974a,b, 1980a,b; Pagano *et al.*, 1974; Poste *et al.*, 1976; Pagano & Weinstein, 1978; Fraley *et al.*, 1979, 1980; Poste, 1980; Gregoriadis, 1980; Wong *et al.*, 1980; Nicolau & Sene, 1982; Nicolau *et al.*, 1983a). Fusogenic agents such as poly(ethylene

glycol) are often not used in liposome-mediated transfer. Various types of vesicles have been described (for references see Straubinger & Papahadjopoulos, 1983) but of these the large unilamellar vesicles are the best suited for transfer as they exhibit a relatively high ratio of internal aqueous space per unit lipid and because they have a higher delivery ratio. The mechanism by which the content of the vesicles is transferred to cells is still unknown, although endocytosis seems to be an important pathway of uptake (for references see Finkelstein & Weissmann, 1978; Straubinger *et al.*, 1983). Fusion of liposomes with plasma membrane is now believed to be a rare event (Szoka *et al.*, 1981). Liposomes containing colloidal gold have been shown to be bound to coated pits, intracellular coated and uncoated vesicles and secondary lysosomes, including dense bodies (Straubinger *et al.*, 1983). These observations emphasize the topographical difficulties encountered with DNA in gene transfer experiments and may account for the low efficiency of transformation achieved with this technique as compared with direct nuclear microinjection (see below). Efforts are now being devoted to devise experimental conditions which may promote the escape of encapsulated molecules from the endocytic pathway (Straubinger *et al.*, 1983).

Liposomes have been used to introduce proteins (Gregoriadis & Buckland, 1973; Gad *et al.*, 1979; Eytan & Eytan, 1980), DNA (Fraley *et al.*, 1980, 1981; Papahadjopoulos *et al.*, 1980a,b; Nicolau &

Sene, 1982; Schäfer-Ridder *et al.*, 1982), RNA (Dimitriadis, 1978; Ostro *et al.*, 1978, 1980; Wilson *et al.*, 1979), lipids (Martin & McDonald, 1976; Struck & Pagano, 1980), metaphase chromosomes (Mukherjee *et al.*, 1978) and small molecules (for references see Papahadjopoulos, 1979; Nicolau & Gersonde, 1979; Gersonde & Nicolau, 1979) into a variety of cultured cells (no cell restriction). This technique, however, is less efficient than the direct microinjection for the injection of RNA (Table 1) or proteins. Likewise, the efficiency of the liposome-mediated gene transfer, and expression, does not exceed that of more conventional methods (Table 1). The technique, however, has the advantage that it can be used with cells that are refractory to transformation by the calcium phosphate technique.

From the above observations it would seem that future applications of this technique will rest mainly on the possibility of delivering drugs (for references see Gregoriadis *et al.*, 1982; Gregoriadis, 1983, 1984) and genetic material (for references see Nicolau *et al.*, 1982, 1983a,b) into cells of living organisms. Techniques are now being developed to improve the specificity of delivery of liposomes by coupling specific antibodies to their surface (targeted liposomes) (for references see Heath *et al.*, 1981, 1983; Barbet *et al.*, 1981; Martin *et al.*, 1981; Martin & Papahadjopoulos, 1982; Huang *et al.*, 1983; Bragman *et al.*, 1983).

Reconstituted Sendai virus envelopes

In this technique the macromolecules to be injected or transplanted are first trapped within the viral envelopes or incorporated into the vesicle membrane during their reconstitution. Fusion of the loaded reconstituted vesicles with the plasma membrane of recipient cells is then promoted by the presence of the virus F protein (fusion factor) in the reconstituted vesicles (Fig. 4) (for references see Uchida *et al.*, 1977; Volsky *et al.*, 1979; Loyter & Volsky, 1979, 1982; Vainstain *et al.*, 1983; Beigel & Loyter, 1983; Loyter *et al.*, 1983, 1984). This technique is restricted to cells having receptors for Sendai virus, although efforts are currently being made to couple anti-(cell membrane) antibodies to the viral envelopes so as to extend the use of the technique to receptor-depleted cells (Loyter *et al.*, 1984). Reconstituted Sendai virus envelopes have been used to transfer proteins (Loyter & Volsky, 1979, 1982; Uchida *et al.*, 1980) and DNA (Loyter *et al.*, 1983; Vainstain *et al.*, 1983) into cultured cells as well as to transplant membrane proteins (Volsky *et al.*, 1979, 1980; Loyter & Volsky, 1982; Beigel & Loyter, 1983; Loyter *et al.*, 1984). This technique does not present any significant advantage over the direct microinjection technique for the injection of proteins (Table 1; immunoglobulin

G), or RNA, but it may be of value for gene-transfer studies (Table 1) for cells that cannot be transfected by using the calcium phosphate technique. Also it is possible that, in the future, this technique may be of use to deliver macromolecules (especially DNA) into cells of living organisms. At present, this technique is mainly being used to transplant membrane proteins into cultured cells to study their degradation and turnover (Beigel & Loyter, 1983).

Recently, Evans & Mayer (1983) described a technique, poly(ethylene glycol)-induced sub-organellar vesicle transplantation, which can also be used to study the degradation of proteins after transplantation of organelles into appropriate target cells. Specifically, this method offers new possibilities for studying the degradation of intracellular proteins that function in defined extracytosolic sites. So far, this technique has been used to study the degradative fate of transplanted total mitochondrial proteins and mitochondrial outer membrane proteins (Evans & Mayer, 1983; Russell & Mayer, 1983; Mayer *et al.*, 1984).

Transfer aided by facilitators

Calcium phosphate technique

This technique, which was originally developed to determine the infectivity of viral DNA (Graham & van der Eb, 1973) is now widely used to assay the transforming activity of viral DNA (for references see Graham, 1977) as well as of DNA extracted from eukaryotic cells (Wigler *et al.*, 1978; Graham *et al.*, 1979; Pellicer *et al.*, 1980). The technique requires the incubation of the recipient cells with coprecipitates of DNA and calcium phosphate. The precipitate binds to the cells and it is then taken up via an endocytotic-like process (Loyter *et al.*, 1982). Intracellularly, the DNA molecules have been shown to be trapped in phagocytic vacuoles and secondary lysosomes (Loyter *et al.*, 1983), but very little is known as to how they gain access to the nucleus and become integrated in the host genome. The technique necessitates the use of specific cell lines such as mouse L cells (Lewis *et al.*, 1980) and NIH 3T3 cells.

To date, this technique has proved invaluable in studies of gene transfer in cultured somatic cells, and it is increasingly being used to transfect genomic clones into target cells. The percentage of stably transformed TK⁺ cells obtained with this technique is comparable with that obtained by microinjection, but many more cells can be transfected at a time. Furthermore, transfection of DNA isolated from neoplastic cells into nonneoplastic recipient cells has provided a unique approach for the study of the genetic control of cancer (for references see Cooper, 1982).

Fig. 4. Schematic representation of the method for using reconstituted Sendai virus envelopes for implantation of membrane protein band 3 into living animal cells

Isolated band 3 is first mixed with a detergent solution of viral envelopes and upon removal of the detergent by co-dialysis, vesicles containing band 3 are formed (VB3). Next, the hybrid vesicles are bound to the recipient cells by incubation at 4°C. Finally, integration takes place following incubation at 37°C. Abbreviations used: HN/F, agglutinin-neuraminidase or fusion glycoprotein of Sendai virus envelopes; B3, human erythrocyte band 3 polypeptide; FELC, Friend erythroleukaemic cells; M, detergent micelle; MM, mixed membrane phospholipid/detergent micelle; SGP, sialoglycoprotein; IP, integral membrane protein. From Loyter *et al.* (1980).

DEAE-dextran

In this method, the DNA is added to the cultured cells in the presence of the polycation DEAE-dextran (Vaheri & Pagano, 1965; Pagano & Vaheri, 1965; McCutchan & Pagano, 1968; Warden & Thorne, 1968; Graham, 1977). The efficiency of transformation (TK^+ ; Table 1) achieved with this technique is considerably lower than that

obtained with other methods currently used for gene transfer (Table 1).

Poly(ethylene glycol)/sucrose shock

This technique is essentially a modification of the procedure of Chu & Sharpe (1981) to transfect cells in suspension. Briefly, after transfection the cells are plated and after 4h they are shocked for

2–4 min with a 60–70% concentration of poly(ethylene glycol) in sucrose. The optimum concentration of poly(ethylene glycol) must be worked out for each individual cell line. This procedure gives excellent results in terms of transient gene expression (phenotypic expression; Table 1) and represents a reasonable alternative to the direct microinjection in those cases in which transfer of genes to a large number of cells is required. The percentage of stably transformed cells (TK⁺; Table 1) obtained with this technique is low.

Osmotic lysis of pinosomes

This technique, which was developed by Okada & Rechsteiner (1982), has been used mainly to introduce proteins (horseradish peroxidase and ricin antibodies) and Dextran 70000 into the cytoplasm of mouse L929 cells. In short, cells are first incubated in the presence of 0.5 M-sucrose/10% poly(ethylene glycol) 1000 and the macromolecules to be injected, followed by a short incubation in hypo-osmotic medium. Under these conditions, the pinocytic vesicles formed during the sucrose treatment release their content as a result of the osmotic lysis.

The technique can be used for large-scale transfer of proteins into cultured mammalian cells and the efficiency of transfer has been shown to be similar to that achieved by the erythrocyte-ghost-mediated transfer (Okada & Rechsteiner, 1982). By the technique of osmotic lysis of pinosomes, it may, however, be possible to transfer larger macromolecules. The technique requires the use of cultured cells exhibiting a high rate of fluid-phase pinocytosis (Steinman *et al.*, 1974; Okada & Rechsteiner, 1982).

Conclusion

In general, the various techniques described in this short Review have opened the possibilities of studying complex biological phenomena that are not amenable to experimentation with conventional systems *in vitro*. The technique of direct microinjection of cultured somatic cells with micropipettes is perhaps the most versatile of the transfer techniques, and, despite its disadvantages, it has brought us closer to the final goal of using the somatic cell as a test tube.

The applications of the various transfer techniques in basic research are numerous and evident (see the text). In medicine, techniques such as the liposome-mediated transfer and the Sendai virus reconstituted envelopes may provide a realistic basis for important applications in areas such as gene transfer (genetic engineering *in vivo*) and delivery of drugs to specific cell types in living organisms.

I would like to thank A. Graessmann, K. Kaltoft, and A. Loyter for providing photographs, and R. Garrett for comments on the manuscript. The work in the author's laboratory was supported by grants from Euratom, the Danish Medical and Natural Research Councils, the Danish Cancer Foundation, NOVO, Carlsberg, and the Aarhus University Research Fund.

References

- Anderson, W. F., Deisseroth, A., Nienhuis, A. W., Gopalakrishnan, T. V., Huang, A. & Krueger, L. (1977) *Nat. Cancer Inst. Monogr.* **48**, 65–73
- Anderson, W. F., Killos, L., Sanders-Haigh, L., Kretschmer, P. J. & Diacumakos, E. G. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5399–5403
- Ansoerge, W. (1982) *Exp. Cell Res.* **140**, 31–37
- Antman, K. H. & Livingston, D. M. (1980) *Cell* **19**, 627–635
- Barbet, J., Machy, P. & Leserman, L. D. (1981) *J. Supramol. Struct. Cell. Biochem.* **16**, 243–258
- Baserga, R., Croce, C. & Rovera, G. (eds.) (1980) *Introduction of Macromolecules into Viable Mammalian Cells*, Alan R. Liss, New York
- Beigel, M. & Loyter, A. (1983) *Exp. Cell Res.* **148**, 95–103
- Bigelow, S., Hough, R. & Rechsteiner, M. (1981) *Cell* **25**, 83–93
- Boogard, C. & Dixon, G. (1983a) *Exp. Cell Res.* **143**, 175–190
- Boogard, C. & Dixon, G. (1983b) *Exp. Cell Res.* **143**, 191–205
- Bragman, K. S., Heath, T. D. & Papahadjopoulos, D. (1983) *Biochim. Biophys. Acta* **730**, 187–195
- Bravo, R. & Celis, J. E. (1980) *Exp. Cell Res.* **126**, 481–485
- Bravo, R., Fey, S. J., Small, J. V., Mose Larsen, P. & Celis, J. E. (1981) *Cell* **25**, 197–202
- Capecci, M. (1980) *Cell* **22**, 479–488
- Capecci, M. R., Vonder Haar, R. A., Capecci, N. E. & Sveda, M. M. (1977) *Cell* **12**, 371–381
- Celis, J. E. (1977) *Brookhaven Symp. Biol.* **29**, 178–196
- Celis, J. E. (1984) *Ciba Found. Symp.* **103**, 220–231
- Celis, J. E. & Bravo, R. (1981) *Trends Biochem. Sci.* **6**, 197–201
- Celis, J. E., Graessmann, M. & Loyter, A. (eds.) (1980a) *Transfer of Cell Constituents into Eukaryotic Cells*, Plenum Press, New York and London
- Celis, J. E., Kaltoft, K. & Bravo, R. (1980b) in *Introduction of Macromolecules into Viable Mammalian Cells* (Baserga, R., Croce, C. & Rovera, G., eds.), pp. 99–123, Alan R. Liss, New York
- Celis, J. E., Kaltoft, K., Celis, A., Fenwick, R. & Caskey, C. T. (1980c) in *Nonsense Mutations and tRNA Suppressors* (Celis, J. E. & Smith, J. D., eds.), pp. 254–276, Academic Press, London
- Chin, D. T., Khuel, L. & Rechsteiner, M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5857–5861
- Chu, G. & Sharpe, P. A. (1981) *Gene* **13**, 197–202
- Cooper, G. M. (1982) *Science* **218**, 801–806
- Diacumakos, E. G. (1973) *Methods Cell Biol.* **8**, 287–311
- Dimitriadis, G. T. (1978) *Nature (London)* **274**, 923–924
- Evans, P. J. & Mayer, R. J. (1983) *Biochem. J.* **216**, 151–161

- Evered, D. & Whelan, J. (eds.) (1984) *Cell Fusion: Ciba Found. Symp.* **103**
- Eytan, G. & Eytan, E. (1980) *J. Biol. Chem.* **255**, 4992–4995
- Feramisco, J. R. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3967–3971
- Finkelstein, M. & Weissmann, G. (1978) *J. Lipid Res.* **18**, 289–303
- Fraley, R. T., Fornari, C. S. & Kaplan, S. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3348–3353
- Fraley, R., Subramani, S., Berg, P. & Papahadjopoulos, D. (1980) *J. Biol. Chem.* **255**, 10431–10435
- Fraley, R., Straubinger, R. M., Rule, G., Springer, E. L. & Papahadjopoulos, D. (1981) *Biochemistry* **20**, 6978–6987
- Furusawa, M. (1980) *Int. Rev. Cytol.* **62**, 29–67
- Furusawa, M., Nishimura, T., Yamaizumi, M. & Okada, Y. (1974) *Nature (London)* **249**, 449–450
- Furusawa, M., Yamaizumi, M., Nishimura, T., Uchida, T. & Okada, Y. (1976) *Methods Cell Biol.* **14**, 73–80
- Gad, A., Broza, R. & Eytan, G. (1979) *FEBS Lett.* **102**, 230–234
- Gersonde, K. & Nicolau, C. (1979) *Blut* **39**, 1–7
- Glacy, S. D. (1983) *J. Cell Biol.* **97**, 1207–1213
- Godfrey, W., Dol, B., Wallace, E. F., Bredt, B. & Wofsy, L. (1981) *Exp. Cell Res.* **135**, 137–146
- Godfrey, W., Dol, B. & Wofsy, L. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2267–2271
- Graessmann, A. (1968) Doctorial Dissertation, Free University of Berlin
- Graessmann, A. & Graessmann, M. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 527–532
- Graessmann, M. & Graessmann, A. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 366–370
- Graessmann, M. & Graessmann, A. (1982) *EMBO J.* **1**, 1081–1088
- Graessmann, M. & Graessmann, A. (1983a) *Methods Enzymol.* **101**, 484–492
- Graessmann, A. & Graessmann, M. (1983b) in *Gene Expression in Normal and Transformed Cells* (Celis, J. E. & Bravo, R., eds.), pp. 247–261, Plenum, New York
- Graessmann, M. & Graessmann, A. (1984) *Miami Winter Symp.*, in the press
- Graessmann, A., Graessmann, M., Hoffmann, E., Niebel, J., Brandner, G. & Mueller, N. (1974) *FEBS Lett.* **39**, 249–251
- Graessmann, A., Graessmann, M. & Mueller, C. (1976) *J. Virol.* **17**, 854–858
- Graessmann, A., Graessmann, M., Guhl, E. & Mueller, C. (1978) *J. Cell Biol.* **77**, 1–8
- Graessmann, M., Graessmann, A. & Mueller, C. (1979a) *Biochem. Biophys. Res. Commun.* **88**, 428–432
- Graessmann, A., Graessmann, M. & Mueller, C. (1979b) *Curr. Top. Microbiol. Immunol.* **81**, 1–21
- Graessmann, A., Graessmann, M. & Mueller, C. (1980a) *Methods Enzymol.* **65**, 816–825
- Graessmann, A., Graessmann, M., Tjian, R. & Topp, W. C. (1980b) *J. Virol.* **33**, 1182–1191
- Graessmann, A., Wolf, H. & Bornkamm, G. W. (1980c) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 433–436
- Graessmann, A., Graessmann, M. & Mueller, C. (1981) *Adv. Cancer Res.* **35**, 111–146
- Graham, F. L. (1977) *Adv. Cancer Res.* **25**, 1–51
- Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467
- Graham, F. L., Bachetti, S. & McKinnon, R. (1979) in *Introduction of Macromolecules into Viable Mammalian Cells* (Baserga, R., Croce, C. & Rovera, G., eds.), pp. 3–25, Alan R. Liss, New York
- Gregoriadis, G. (1980) in *Transfer of Cell Constituents into Eukaryotic Cells* (Celis, J. E., Graessmann, A. & Loyter, A., eds.), pp. 173–199, Plenum, New York
- Gregoriadis, G. (1983) *Trends Pharmacol. Sci.* **4**, 304–307
- Gregoriadis, G. (ed.) (1984) *Liposome Technology*, CRC Press, Boca Raton, FL, in the press
- Gregoriadis, G. & Allison, A. C. (eds.) (1980) *Liposomes in Biological Systems*, John Wiley and Sons, New York
- Gregoriadis, G. & Buckland, R. A. (1973) *Nature (London)* **244**, 170–172
- Gregoriadis, G., Senior, J. & Trowet, A. (eds.) (1982) *Targeting of Drugs*, Plenum, New York
- Heath, T. D., Fraley, R. T. & Papahadjopoulos, D. (1981) *Science* **210**, 599–601
- Heath, T. D., Montgomery, J. A., Piper, J. A. & Papahadjopoulos, D. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1377–1381
- Huang, A., Kennel, S. J. & Huang, L. (1983) *J. Biol. Chem.* **258**, 14034–14040
- Hudziak, R. M., Laske, F. A., RajBhandary, U. L., Sharp, P. A. & Capecchi, M. (1982) *Cell* **31**, 137–146
- Iino, T., Furusawa, M., Furusawa, I. & Obinata, M. (1983) *Exp. Cell Res.* **148**, 475–480
- Kaltoft, K. & Celis, J. E. (1978) *Exp. Cell Res.* **115**, 423–428
- Kaltoft, K., Zeuthen, J., Engbaek, F., Piper, P. & Celis, J. E. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2793–2797
- Katznelson, R. & Kulka, R. G. (1983) *J. Biol. Chem.* **258**, 9597–9600
- Klimkowsky, M. W. (1981) *Nature (London)* **291**, 249–251
- Kopchick, J. J. & Stacey, D. W. (1984) *Mol. Cell. Biol.*, in the press
- Kopchick, J. J., Ju, G., Skalka, A. M. & Stacey, D. W. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4383–4387
- Kreis, T. E., Winterhalter, K. H. & Birchmeier (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3814–3818
- Krieger, M. P., Griffin, J. D. & Livingston, D. M. (1978) *Cell* **14**, 983–994
- Kulka, R. G. & Loyter, A. (1979) *Curr. Top. Membr. Transport* **12**, 365–430
- Lewis, W. H., Srinivasan, P. R., Stokes, N. & Siminovich, L. (1980) *Somatic Cell Genet.* **6**, 333–347
- Lin, J. J.-C. & Feramisco, J. R. (1981) *Cell* **24**, 185–193
- Loyter, A. & Volsky, D. J. (1979) in *Membrane Biogenesis* (Lee, S. P., Schatz, G. & Ernster, L., eds.), pp. 319–330, Addison-Wesley, London
- Loyter, A. & Volsky, D. J. (1982) *Cell Surface Reviews* (Poste, G. & Nicolson, G. L., eds.), pp. 215–266, North-Holland Publishing Co., Amsterdam
- Loyter, A., Zakai, N. & Kulka, R. G. (1975) *J. Cell Biol.* **66**, 292–304
- Loyter, A., Volsky, D. J., Beigel, M., Ginsburg, H. & Cabantchik, Z. I. (1980) in *Transfer of Cell Constituents into Eukaryotic Cells* (Celis, J. E., Graessmann, A. & Loyter, A., eds.), pp. 143–154, Plenum, New York

- Loyter, A., Scangos, G., Juricek, D. & Keene, D. (1982) *Exp. Cell Res.* **139**, 223–234
- Loyter, A., Vainstain, A., Graessmann, M. & Graessmann, A. (1983) *Exp. Cell Res.* **143**, 415–425
- Loyter, A., Tomasi, M., Gitman, A. G., Etinger, L. & Nussbaum, O. (1984) *Ciba Found. Symp.* **103**, 163–175
- Martin, F. & McDonald, R. (1976) *J. Cell Biol.* **70**, 515–526
- Martin, F. J. & Papahadjopoulos, D. (1982) *J. Biol. Chem.* **257**, 286–288
- Martin, F. J., Hubbell, W. L. & Papahadjopoulos, D. (1981) *Biochemistry* **20**, 4229–4238
- Mayer, R. J., Evans, P., Russell, S. & Amenta, J. S. (1984) *Ciba Found. Symp.* **103**, 202–214
- McCutchan, J. H. & Pagano, J. S. (1968) *J. Natl. Cancer Inst.* **41**, 351–357
- McGarry, T., Hough, R., Rogers, S. & Rechsteiner, M. (1983) *J. Cell Biol.* **96**, 338–346
- Mercer, E. W., Nelson, D., Hyland, J. K., Croce, C. & Baserga, R. (1983) *Virology* **127**, 149–158
- Mueller, C., Graessmann, A. & Graessmann, M. (1978) *Cell* **15**, 579–585
- Mukherjee, A. B., Orloff, S., Butler, J. D., Triche, T., Lalley, P. & Schulman, J. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1361–1365
- Neff, N. T., Bourret, L., Miao, P. & Dice, J. F. (1981) *J. Cell Biol.* **91**, 184–194
- Nicolau, C. & Gersonde, K. (1979) *Naturwissenschaften* **66**, 561–564
- Nicolau, C. & Sene, C. (1982) *Biochim. Biophys. Acta* **721**, 185–190
- Nicolau, C., Le Pape, A., Soriano, P., Fargette, D., Juhel, M. F. & Muh, J. P. (1982) in *Proceedings of the FEBS Meeting on Cell Differentiation* (Evangelopoulos, A. E., ed.), pp. 321–331, Alan R. Liss, New York
- Nicolau, C., Soriano, P., Le Pape, A., Londos-Gagliardi, D., Legrand, A. & Grosse, E. (1983a) *Biol. Cell.* **47**, 121–130
- Nicolau, C., Le Pape, A., Soriano, P., Fargette, F. & Juhel, M. F. (1983b) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1068–1072
- Okada, C. Y. & Rechsteiner, M. (1982) *Cell* **29**, 33–41
- Ostro, M. J., Giacomoni, D. & Dray, S. (1980) in *Introduction of Macromolecules Into Viable Cells* (Baserga, R., Croce, C. & Rovera, G., eds.), pp. 239–259, Alan R. Liss, New York
- Ostro, M. C., Giacomini, D., Lavelle, D., Paxton, W. & Dray, S. (1978) *Nature (London)* **244**, 921–923
- Pagano, J. S. & Vaheri, A. (1965) *Arch. Gesamte Virusforsch.* **17**, 456–464
- Pagano, R. E. & Weinstein, J. N. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 435–468
- Pagano, R., Huang, L. & Wey, C. (1974) *Nature (London)* **252**, 166–167
- Papahadjopoulos, D. (1979) *Annu. Rep. Med. Chem.* **14**, 250–260
- Papahadjopoulos, D., Mayhew, R. & Poste, G. (1974a) *Nature (London)* **252**, 163–166
- Papahadjopoulos, D., Poste, G. & Mayhew, E. (1974b) *Biochim. Biophys. Acta* **363**, 404–418
- Papahadjopoulos, D., Fraley, R. & Heath, T. (1980a) in *Liposomes and Immunobiology* (Tom, B. & Six, H., eds.), pp. 151–164, Elsevier/North-Holland, New York
- Papahadjopoulos, D., Wilson, T. & Taber, R. (1980b) in *Transfer of Cell Constituents into Eukaryotic Cells* (Celis, J. E., Graessmann, A. & Loyter, A., eds.), pp. 155–172, Plenum, New York
- Paraf, A. & Nicolau, C. (eds.) (1981) *Liposomes in Study of Drug Activity and Immunocompetent Cell Function*, Academic Press, New York
- Pellicer, A., Robins, B., Wold, R., Sweet, J., Jackson, I., Lowry, J. M., Roberts, G. K., Sim, S., Silverstein, S. & Axel, R. (1980) *Science* **209**, 1414–1422
- Poste, G. (1980) in *Liposomes in Biological Systems* (Gregoriadis, G. & Allison, A., eds.), pp. 101–151, John Wiley and Sons, New York
- Poste, G., Papahadjopoulos, D. & Vail, W. J. (1976) *Methods Cell Biol.* **14**, 33–71
- Pugatsch, T. & Stacey, D. W. (1982) *J. Virol.* **43**, 503–510
- Rassoulzadegan, M., Binetruy, B. & Cuzin, F. (1982) *Nature (London)* **295**, 257–259
- Rechsteiner, M. C. (1982) in *Techniques in Somatic Cell Genetics* (Shay, J. W., ed), pp. 385–398, Plenum Press, New York
- Rechsteiner, M. & Kuehl, L. (1979) *Cell* **16**, 901–908
- Rechsteiner, M., Chin, D., Hough, R., McGarry, T., Rogers, S., Rote, K. & Wu, L. (1984) *Ciba Found. Symp.* **103**, 181–195
- Russell, S. & Mayer, R. J. (1983) *Biochem. J.* **216**, 163–175
- Schäffer-Ridder, M., Wang, Y. & Hofschneider, P. H. (1982) *Science* **215**, 166–168
- Schlegel, R. A. & Mercer, W. E. (1980) in *Introduction of Macromolecules into Viable Mammalian Cells* (Baserga, R., Croce, C. & Rovera, G., eds.), pp. 145–155, Alan R. Liss, New York
- Schlegel, R. A. & Rechsteiner, M. (1975) *Cell* **5**, 371–379
- Schlegel, R. A. & Rechsteiner, M. C. (1978) *Methods Cell Biol.* **20**, 341–354
- Shen, Y., Hirschhorn, R. R., Mercer, W. E., Surmacz, E., Tsutsui, Y., Soprano, K. J. & Baserga, R. (1982) *Mol. Cell. Biol.* **2**, 1145–1154
- Stacey, D. W. (1980a) *Cell* **21**, 811–820
- Stacey, D. W. (1980b) in *Transfer of Cell Constituents into Eukaryotic Cells* (Celis, J. E., Graessmann, A. & Loyter, A., eds.), pp. 29–60, Plenum, London
- Stacey, D. W. (1981) *Methods Enzymol.* **79**, 76–78
- Stacey, D. W. & Allfrey, V. G. (1976) *Cell* **9**, 725–732
- Stacey, D. W. & Allfrey, V. G. (1977) *J. Cell Biol.* **75**, 807–817
- Stacey, D. W., Allfrey, V. G. & Hanafusa, H. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1614–1618
- Steinman, R. M., Silver, J. M. & Cohn, Z. A. (1974) *J. Cell Biol.* **63**, 949–969
- Straubinger, R. M. & Papahadjopoulos, D. (1983) *Methods Enzymol.* **101**, 512–527
- Straubinger, R. M., Hong, K., Friend, D. S. & Papahadjopoulos, D. (1983) *Cell* **32**, 1069–1079
- Struck, D. & Pagano, R. (1980) *J. Biol. Chem.* **255**, 5404–5410
- Szoka, F., Magnusson, K.-E., Wojcieszyn, J., Hou, Y., Derzko, Z. & Jacobsen, K. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1685–1689
- Timm, B., Kondor-Koch, C., Lehrach, H., Reidel, H., Edström, J. E. & Garoff, H. (1983) *Methods Enzymol.* **96**, 496–511

- Tjian, R., Fey, G. & Graessmann, A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1279–1283
- Uchida, T., Yamaizumi, M. & Okada, Y. (1977) *Nature (London)* **266**, 839–840
- Uchida, T., Yamaizumi, M., Mekada, E. & Okada, Y. (1980) in *Introduction of Macromolecules into Viable Mammalian Cells* (Baserga, R., Croce, C. & Rovera, G., eds.), pp. 169–185, Alan R. Liss, New York
- Vainstain, A., Razin, A., Graessmann, A. & Loyter, A. (1983) *Methods Enzymol.* **101**, 492–512
- Vaheri, A. & Pagano, J. S. (1965) *Virology* **27**, 435–436
- Volsky, D. J., Cabantchik, Z. I., Beigel, M. & Loyter, A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5440–5444
- Volsky, D. J., Shapiro, I. M. & Klein, G. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5453–5457
- Warden, D. & Thorne, H. V. (1968) *J. Gen. Virol.* **3**, 371–377
- Wassermann, M., Zakai, N., Loyter, A. & Kulka, R. G. (1976) *Cell* **7**, 551–556
- Wehland, J. & Weber, K. (1980) *Exp. Cell Res.* **127**, 397–408
- Wiberg, F. C., Sunnerhagen, P., Kaltoft, K., Zeuthen, J. & Bjursell, G. (1983) *Nucleic Acid Res.* **11**, 7287–7302
- Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1978) *Cell* **14**, 725–731
- Wilson, T., Papahadjopoulos, D. & Taber, R. (1979) *Cell* **17**, 77–84
- Wong, T. K., Nicolau, C. & Hofschneider, P. H. (1980) *Gene* **10**, 87–94
- Wu, R., Grossman, L. & Moldave, K. (eds.) (1983) *Methods Enzymol.* **101C**
- Yamaizumi, M., Uchida, T., Mekada, E. & Okada, Y. (1979) *Cell* **18**, 1009–1014
- Yamamoto, F. & Furusawa, M. (1978) *Exp. Cell Res.* **117**, 441–445
- Zavortink, M., Thacher, T. & Rechsteiner, M. C. (1979) *J. Cell Physiol.* **100**, 175–186