

## COMPOSITION AND METHODS FOR TRANSFECTING EUKARYOTIC CELLS

This application is a continuation of U.S. patent application Ser. No. 08/274,397, filed Jul. 12, 1994, now U.S. Pat. No. 5,578,475, which is a continuation-in-part of U.S. patent application Ser. No. 08/090,290, filed Jul. 12, 1993, now abandoned, both of which are incorporated by reference in their entirety herein.

### FIELD OF THE INVENTION

Compositions of cationic lipids and viral components useful for transfecting eukaryotic cells with nucleic acids and for introduction of other macromolecules into such cells are disclosed. Also disclosed are methods of transfecting eukaryotic cells employing such compositions.

### BACKGROUND OF THE INVENTION

Lipid aggregates such as liposomes can function to facilitate introduction of macromolecules, such as DNA, RNA, and proteins, into living cells. Recently, it has been shown that lipid aggregates comprising cationic lipid components can be especially effective for delivery and introduction of large anionic molecules, such as nucleic acids, into certain types of cells. See Felgner, P. L. and Ringold, G. M. (1989) *Nature* 337:387-388. Since the membranes of most cells have a net negative charge, anionic molecules, particularly those of high molecular weight, are not readily taken up by cells. Cationic lipids aggregate to and bind polyanions, such as nucleic acids, tending to neutralize the negative charge. The effectiveness of cationic lipids in transfection of nucleic acids into cells is thought to result from an enhanced affinity of cationic lipid-nucleic acid aggregates for cells.

A variety of types of lipid aggregates are known, including liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, having particle sizes in the nanometer to micrometer range. As is well-known in the art, the structures of lipid aggregates depend on the lipid composition and the method employed to form the aggregate. Cationic lipids can be used alone or in combination with non-cationic lipids, for example, with neutral phospholipids like phosphatidylethanolamines, to form positively charged vesicles and other lipid aggregates which are able to bind nucleic acids. The positively charged lipid aggregates bind to nucleic acids, can then be taken up by target cells and thus facilitate transfection of the target cells with the nucleic acid. (See, Felgner, P. L. et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7417; Epstein, D. et al. U.S. Pat. No. 4,897,355.)

Cationic lipids are not universally effective for transfection of all cell types. Effectiveness of transfection of different cells depends on the cationic lipid composition used and the type of lipid aggregate formed. In addition, a particular cationic lipid may be more or less toxic to a given cell line, limiting the type of concentration of lipid that can be employed for transfection. Certain types of higher eukaryotic cells are not readily transfected employing presently available cationic lipids. These hard-to-transfect cells generally include suspension cell lines and primary human cell lines and, more specifically, include fibroblasts and macrophage cell lines. Compositions and methods which would generally enhance the efficiency of cationic lipid-mediated transfection and/or broaden the range of cell types that can be efficiently transfected with cationic lipid-DNA complexes would represent valuable improvements in the art.

Many biological materials are taken up by cells by receptor-mediated endocytosis. See: Pastan and Willingham

(1981) *Science* 214:504-509. This mechanism involves binding of a ligand to a cell-surface receptor, clustering of ligand-bound receptors, and formation of coated pits followed by internalization of the ligands into endosomes. Both enveloped viruses, like influenza virus and alphaviruses, and non-enveloped viruses, like adenovirus, infect cells via endocytotic mechanisms. See: Pastan, I. et al. (1986) in *Virus Attachment and Entry into Cells*, (Crowell, R. L. and Lonberg-Holm, K., eds.) Am. Soc. Microbiology, Washington, p. 141-146; Kielian, M. and Helenius, A. (1986) "Entry of Alphaviruses" in *The Togaviridae and Flaviviridae*, (Schlesinger, S. and Schlesinger, M. J., eds.) Plenum Press, New York p. 91-119; FitzGerald, D. J. P. et al. (1983) *Cell* 32:607-617. Receptor-mediated endocytosis has been exploited to deliver DNA into cells. Wu, G. Y. and Wu, C. H. (1987) *J. Biol. Chem.* 262:4429-4432; Wagner, E. et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:3410-3414. These methods employ bifunctional conjugates having a ligand, which binds to a specific cell-surface receptor, covalently linked to a DNA-binding domain. Asialoglycoprotein-polylysine conjugates and human transferrin-polylysine conjugates have, for example, been demonstrated to mediate DNA entry into certain eukaryotic cells. (Wagner, E. et al., 1990, *supra*).

Curiel, D. T. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8850-8854 and Cotton, M. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6094-6098 have recently reported that receptor-mediated transfection via transferrin-polylysine/DNA complexes is enhanced by simultaneously exposing the cells to defective adenovirus particles. These authors report that adenovirus particles function to disrupt endosomes containing the viral particle and the DNA complex. Replication-defective adenovirus particles and psoralen inactivated adenovirus were reported to enhance transfection. Adenovirus enhancement of transfection is limited, however, to cells which have both a ligand receptor, i.e., transferrin receptor, and an adenovirus receptor. Direct coupling of polylysine/DNA complexes to adenoviruses has also been employed for transfection. Curiel, D. T. et al. (1992) *Hum. Gene Therapy* 3:147-154; Wagner, E. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6099-6103. In related work, Wagner, E. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7934-7938, report augmentation of transfection in several cell lines when hemagglutinin HA-2 N-terminal fusogenic peptides from influenza virus are included in transferrin-polylysine-DNA complexes. The use of influenza peptide conjugates was, however, reported to be less effective for enhancement of transfection than defective adenovirus.

PCT patent applications WO 93/07283 and WO 93/07282, both published Apr. 15, 1993, relate to transfection of higher eukaryotic cells via ligand/polylysine/DNA complexes and endosomolytic agents, such as adenovirus and HA-2 fusogenic peptides.

Alphaviruses, mosquito-transmitted members of the family *Togaviridae*, are RNA-containing enveloped viruses (also called membrane viruses). Alphaviruses include, among others, Sindbis and Semliki Forest (SFV) viruses, several equine encephalitis viruses (Eastern (EEE), Western (WEE) and Venezuelan (VEE)), Chikungunya virus and Ross River virus. Sindbis and Semliki Forest viruses are the least virulent and best characterized alphaviruses. See generally: Schlesinger, S. and Schlesinger, M. J., eds. (1986) *The Togaviridae and Flaviviridae*, Plenum Press. Alphaviruses in general, and specifically SFV and Sindbis virus, have very broad host ranges. SFV infects a wide variety of cultured cells including mammalian (human, monkey,