Electrofusion and Electroporation of Plants

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ABSTRACT

The utilization of electrofusion and electroporation techniques has had a major impact on the genetic manipulation of plants within the last decade. This review of the development of electrofusion and electroporation, as it applies to plants, highlights major developmental aspects of this technology. These include mechanisms for cell fusion, molecular exchange, and parameters that affect the efficiency of fusion and electroporation.

Electrofusion (electric field-induced cell-to-cell fusion) and electroporation (electric field-mediated membrane permeabilization) are simple procedures that may be used for altering the genetic make-up of organisms. Both procedures induce transient, unstable regions in membranes using short, high-voltage electrical fields. Electric field-induced fusion avoids many of the disadvantages of chemical-, mechanical-, and viral-induced fusion procedures such as cell toxicity, host range limitations, or a limitation on the number of cells that can be fused. Electrofusion has the disadvantage common to all somatic fusion procedures: it combines two or more genomes so that the hybrid has a polyploid chromosome number. The stabilization of the hybrid chromosome number and the evaluation of the loss of extraneous genes are postelectrofusion analyses that are needed in each experiment.

Electrofusion was first reported by Senda et al. (18) in 1979. They fused protoplasts of Rauwolfia serpentina var Bentham by bringing two glass capillary microelectrodes in contact with the adhering protoplasts and applying an electric impulse. Although this method had a limited yield because only two cells at a time could be fused, it represented an important milestone in the development of the electrofusion technique. Pioneering research in electrofusion was also provided by Zimmermann and coworkers (22, 23) when they demonstrated that plant protoplasts from Vicia faba mesophyll cells could be electrofused in large numbers in a 1-mL fusion chamber with parallel electrodes. They coupled electrofusion with dielectrophoresis (12), a process that brings cells in close membrane contact by the application of a low voltage AC.¹

During dielectrophoresis, charged dipoles induced in the cells when a weak AC current passes through the membrane suspension cause the cells between the electrodes to become aligned in chain-like aggregates, often referred to as "pearl chains." It has been suggested that the AC field modifies numerous membrane molecules and ultimately causes the formation of lipid domains by the migration of protein subunits within the cell membrane (23). Subsequent application of a higher strength DC pulse(s) results in the reversible breakdown of the cell membrane (electrical breakdown) and the formation of pores in the zone of membrane contact. Fusion is most effective when the direction of the field is perpendicular to the plane of the cell membranes that are in contact. The membrane breakdown is asymmetric, with pores first forming at the charged poles, and pore formation then spreading over the entire membrane surface (5).

Electrical breakdown is the primary process responsible for the initiation of fusion. The process is reversible, but the recovery time may vary among different membrane types (20). In any case, electrical breakdown is irreversible if either the field strength or the pulse duration exceed critical threshold levels (23). Effective electrical breakdown can occur through a variety of combinations of field strengths and pulse durations when one is increased while the other is decreased. The mechanism of fusion is unclear, however; it may occur when random collision of free hydrophobic edges of the pores in separate membranes result in membrane continuity.

Electrofusion can also be induced when the DC pulse is applied to cells first and then the membranes are brought in contact with an AC field (20). This procedure is referred to as the PF protocol, whereas the original procedure of dielectrophoresis followed by the fusion pulse is referred to as the CF protocol. Fusion nearly always coincides with the application of a pulse when the CF protocol is used. When the PF protocol is used, fusion occurs only when the membranes are brought into contact, reflecting the presence of long-lived fusogenic membrane modifications. Fusion yields are generally higher using the CF protocol, probably due to the large peak diameter of electropores present when membranes are in close contact, compared with the residual pores that would be present at the time of contact when the PF protocol is used.

Early skepticism regarding the viability of cells after electrofusion was dispelled by Bates and Hasenkampf (4) when they produced somatic hybrid plants from electrofused protoplasts of Nicotiana plumbaginifolia and N. tabacum. Hybridity of these plants was confirmed by esterase isozyme pattern analysis. For a review of plant somatic hybrids produced by electrofusion, see Bates (2).

¹ Abbreviations: AC, alternating sine wave current; CAT, chloramphenicol acetyltransferase; CF, "contact-first"; DC, direct current; PF, "pulse-first."
In contrast with electroporation, electric field-mediated DNA transfer offers the possibility for the transfer of small numbers of well-characterized genes into the recipient cell. Electroporation allows the introduction of DNA, RNA, proteins, drugs, and dyes into recipient cells through electropores induced when DC pulses are applied. In 1982, Wong and Neumann (21) established that plasmid DNA could be taken up and expressed by animal cells. Shortly thereafter, electroporation of plants was demonstrated from Fromm et al. (9) when they showed the uptake and expression of DNA in protoplasts from both monocots (maize) and dicots (carrots and tobacco). Recent work has provided transformed rice (fertile) (19) and maize (sterile) (14) plants through electroporation of protoplasts. Callis et al. (6) established that mRNA can be taken up and expressed in eukaryotic cells, providing a rapid and convenient method for analyzing the effects of mRNA's structure on activity in vivo. The expression of RNAs in protoplasts has been visualized by autoradiography (6), histology, and video imaging (10).

Although most studies have used isolated protoplasts, genetic material has been electroporated into other plant cells. For example, Morikawa et al. (11) showed that tobacco mosaic virus RNA could be electroporated into partially digested tobacco mesophyll cells and attempted to differentiate the modified procedure by naming it electroinjection. Other reports have demonstrated that it is possible to electroporate DNA through thin plant cell walls. For example, Dekeyser et al. (7) reported the uptake and expression of DNA by intact leaf segments of rice, wheat, maize, and barley, and Abdul-Baki et al. (1) have reported the uptake and expression of DNA by germinating tobacco pollen.

For both electrofusion and electroporation, the application of high-voltage DC pulses induces the formation of pores in cell membranes. It has been commonly assumed that the mechanism of exchange of molecules across the membrane is diffusional. Recent research by Sowers (20) and Dimitrov and Sowers (8) indicate that an active process, which the authors called electroosmosis, may be the dominating mechanism of molecular exchange. They observed the transfer of fluorescent dyes, fluorescein isothiocyanate-dextran, and N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) glucosamine through electropores induced in erythrocyte ghost membranes when the ghosts were subjected to electrical pulses. The net flow of dye was into the cells at the anode-facing (+) hemisphere and outward at the cathode-facing (−) hemisphere. This unidirectional molecular exchange was faster than could be accounted for by diffusion alone (8).

Both electrofusion and electroporation require the optimization of parameters related to the electrical pulse, including field strength and pulse duration. These parameters need to be adjusted for a given cell type and are dependent on the type of the pulse wave generator that is used. Saunders et al. (17) found differences in the uptake and expression of cucumber mosaic virus RNA by tobacco protoplasts using both square wave and exponential wave pulse generators. When both machines were optimized for maximal performance, the square wave generator had a much broader range of experimental conditions that led to the expression of cucumber mosaic virus RNA. The exponentially decaying wave generator gave high rates of both uptake and expression; however, the pulse field strength working range was very narrow. Regardless of which wave generator is used, it is clear that the experimental protocol must be optimized for each cell type that is being examined. The optimization often involves the use of different electroporation chambers. The cuvette-style electroporation chamber (13) increases the ease and simplicity in the handling of cells during electroporation and has evolved as an industry standard.

The use of additives in the electroporation/electrofusion media has brought mixed results. Increased efficiency in the fusion of animal cells was produced by pretreatments with proteolytic enzymes (23). Ruzin and McCarthy (16) found protease, pronase, and trypsin to have positive, but not profound, effects on the electrofusion of tobacco protoplasts. Presumably, proteolytic enzymes enhance the emergence of protein-free lipid membranes produced by the AC field. It is likely that these enzymes are less effective in plant protoplast fusion because plant protoplasts are usually isolated using relatively crude commercial enzyme solutions that may already contain proteolytic enzymes.

The physical form of the DNA appears to be important for efficient electroporation of plant protoplasts. Bates et al. (3) report similar levels of CAT expression supported by linear and supercoiled forms of pCaMVCATM in electroporated carrot protoplasts. They also provide evidence for the ligation and recircularization of linear plasmids following electroporation into carrot protoplasts. Rodenburg et al. (15) found that single-stranded DNA yielded a higher frequency of stable kanamycin-resistant transformants than double-stranded DNA when N. tabacum protoplasts were electroporated in the presence of single-stranded or double-stranded M13 DNA carrying the neomycin phosphotransferase II and CAT genes. It is possible that single-stranded DNA enters the nucleus more efficiently than double-stranded DNA, or integrates into the plant genome at a higher frequency. Interestingly, both complementary strands and double-stranded DNA gave rise to a similar level of CAT activity in transient assays. This can be explained by assuming that single-stranded DNA is rapidly converted into double-stranded DNA in plant cells.

The area of electrofusion and electroporation of plant cells is rapidly expanding. Both techniques present the plant scientist with powerful tools for gene transfer. Further modifications and improvements of these technologies should extend their gene transfer capability to a broad range of plants. Electroporation, in particular, promises the transfer of discrete gene packages to virtually any plant species through the use of intact cells or other plant parts.

**LITERATURE CITED**