High-Efficiency Transformation of *Chlamydomonas reinhardtii* by Electroporation

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ABSTRACT

We have established a high-efficiency method for transforming the unicellular, green alga *Chlamydomonas reinhardtii* by electroporation. Electroporation of strains CC3395 and CC425, cell wall-less mutants devoid of argininosuccinate lyase (encoded by *ARG7*), in the presence of the plasmid pJD67 (which contains *ARG7*) was used to optimize conditions for the introduction of exogenous DNA. The conditions that were varied included osmolarity, temperature, concentration of exogenous DNA, voltage and capacitance. Following optimization, the maximum transformation frequency obtained was 2×10^5 transformants per μg of DNA; this frequency is two orders of magnitude higher than obtained with the current standard method using glass beads to introduce exogenous DNA. The electroporation procedure described in this article is of general utility, and makes it feasible to isolate genes by direct complementation of *Chlamydomonas reinhardtii* mutants.

CHLAMYDOMONAS reinhardtii is a unicellular, eukaryotic green alga that has been used to elucidate aspects of photosynthesis, phototaxis, flagella assembly, cell wall biogenesis, gametogenesis, cell cycle events, mating processes, and nuclear/chloroplast interactions (for review, see Rochaix 1995). There are many advantages of using *C. reinhardtii* as a model eukaryotic, photosynthetic organism: (1) It grows rapidly (doubling time, 6–8 hr) and is easy and inexpensive to culture, (2) it can grow photoautotrophically or heterotrophically, which permits the isolation of mutants unable to perform photosynthesis, (3) it is amenable to classical genetic analysis, (4) it is haploid during vegetative growth, allowing any mutation to be immediately expressed, (5) characterized mutants are available at the *C. reinhardtii* stock center (Harris 1989), (6) exogenous DNA can be introduced into the nuclear, chloroplast, and mitochondria genomes (Boynton *et al.* 1988; Kindle *et al.* 1989; Newman *et al.* 1991; Sodeinde and Kindle 1993; Schnell and Lefebvre 1993; Randolph-Anderson *et al.* 1993; Davies *et al.* 1994, 1996), (7) a reporter gene has been developed and used to dissect the regulation of various promoters (Davies *et al.* 1992; Davies and Grossman 1994; Quinn and Merchant 1995), and (8) cosmid and yeast artificial chromosome libraries have been constructed (Purton and Rochaix 1994; Zhang *et al.* 1994; Infante *et al.* 1995), including an indexed cosmid library (Zhang *et al.* 1994) for the complementation of mutants (Sodeinde and Kindle 1993; Tam and Lefebvre 1993; Zhang *et al.* 1994; Davies *et al.* 1996; Vashishtha *et al.*1996; Funke *et al.* 1997).

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One of the recent technological advances that has helped to establish *C. reinhardtii* as a model photosynthetic organism for the analysis of biological processes has been the development of a transformation system for the stable introduction of DNA into the nuclear genome (Kindle 1990). This method of transformation, superior to other methods that have been used (Kindle *et al.* 1989; Brown *et al.* 1991; Dunahay 1993; Butanaev 1994; Tang *et al.* 1995), involves vortexing cells in the presence of exogenous DNA, acid washed glass beads, and polyethylene glycol. This procedure can yield up to 1000 transformants per μg DNA. However, this frequency is still lower than that observed for various unicellular organisms (*e.g.*, yeast, *E. coli*, and cyanobacteria) where other methods of introducing the exogenous DNA were used. The glass bead transformation procedure was originally developed to introduce DNA into yeast (Costanzo and Fox 1988), and although it is convenient, it generally results in low efficiencies of transformation (~200 transformants/μg DNA). Either electroporation (Manivasakam and Schiestl 1993) or the lithium acetate/single-stranded DNA/polyethylene glycol methods (Gietz *et al.* 1995) can yield transformation frequencies of $\sim 10^6$ transformants/μg DNA in yeast. An electroporation procedure was optimized in the studies presented in this article to yield transformation frequencies far better than those that have been reported previously for *C. reinhardtii*.

MATERIALS AND METHODS

***C. reinhardtii* cultures and growth conditions:** *C. reinhardtii* strains CC125 (wild-type mt+) and CC425 (*arg7-8 cw15 mt+ sr-u-2-60*) were obtained from Dr. J. Davies at the Carnegie Institution of Washington (Stanford, CA), and strain CC3395

(*arg7-8 cwd mt+*) was obtained from Dr. R. Funke at the University of Nebraska, Lincoln. The last strain was originally isolated by Dr. R. Matagne of Liége University (Liége, Belgium). Cells were grown in 100 ml TAP culture medium (Harris 1989) and supplemented with 200 μg/ml arginine when appropriate. Illumination was continuous at 50 μmol photons m^{-2} sec^{-1} from fluorescent tubes. The flasks were agitated on a gyratory shaker (120 rpm) at 27° without aeration.

Preparation of exogenous DNA: The plasmid pJD67, carrying the *ARG7* gene, was obtained from Dr. J. Davies at the Carnegie Institution (Davies *et al.* 1994). The cosmid clone 42G2, which contains the *ARG7* gene, was obtained from Dr. D. Weeks of the University of Nebraska, Lincoln (Zhang *et al.* 1994). These DNAs were amplified in *E. coli* strain JM109, and purified by a standard alkali lysis extraction procedure followed by centrifugation in a cesium chloride gradient (Sambrook *et al.* 1989). Salmon sperm DNA (Sigma, St. Louis, MO) was used as a carrier during transformation; the carrier DNA was dissolved in water at 10 mg/ml, sheared by sonication and then denatured by boiling for 5 min. All of the DNA samples were quantified by measuring the absorbance at 260 nm.

Electroporation protocol: The cell cultures were chilled on ice prior to the addition of a 10% Tween-20 solution at 1/2000 (v/v); this facilitates pelleting of the *C. reinhardtii* cells. The cells were collected by centrifugation at 800 × *g* for 5 min at 4° and resuspended in Tris acetate phosphate (TAP) medium containing indicated concentrations (typically 40 mm) of sucrose to a final density of between 1×10^8 and 4×10^8 cells per ml. Under standard conditions, 10 μg/ml of the plasmid pJD67 (linearized by *Hin*dIII digestion) and 200 μg/ml of carrier DNA were added. The cell suspension of 250 μl was placed into a disposable electroporation cuvette with a 4-mm gap (Bio-Rad Labs., Hercules, CA), which was then immersed in a water bath to maintain specific temperatures. An exponential electric pulse (typically between 1900 and 2400 V/cm) was applied to the sample using the model GTE-10 (SHIMADZU, Kyoto, Japan) electroporation apparatus. Unless otherwise noted, the capacitance was set at 10 μF and no shunt resistor was used. It was crucial that less than 1 hr elapse between the time of harvesting the cells and the application of the electric field. Following electroporation, the cuvette was removed from the electroporation apparatus and incubated in a 25° water bath for at least 5 min (and no more than 60 min), and an aliquot of the cell suspension was plated onto solid medium (TAP-0.5% agarose) by a starch embedding method (described below). Between 0.4% and 2% of the transformation mixture was plated in duplicate to determine transformation frequencies. The plates were illuminated with fluorescent tubes at 80 μmol photons m^{-2} sec^{-1} at between 25–29°. Before optimization of the conditions (Figures 2–5), the frequencies of transformation in each experiment are presented relative to the highest value, which is set at 1.

Starch embedding method: Corn starch was washed sequentially with distilled water and ethanol. The washed starch was stored in 75% ethanol to prevent bacterial contamination. Before each experiment, the ethanol was replaced with TAP-sucrose medium by repeated centrifugations and resuspensions. The starch was finally resuspended to 20% (w/v) in TAP-sucrose medium, and polyethylene glycol 8000 was added to 0.4% (w/v); the latter facilitates smooth, even spreading of the starch over the plate. The starch suspension of 1 ml was spread with an appropriate volume of electroporated cell suspension (0.5–50 μl) over the top of solid medium in a 9-cm-diameter petri plate.

Southern blot analysis: Total DNA was isolated by lysing the *C. reinhardtii* cells in buffer containing 1% SDS, 200 mm $NaClO_4$, 20 mm EDTA, 40 mm Tris-HCl pH 8.0, extracting the lysate with phenol and then precipitating the nucleic acid with ethanol. Isolated DNA (5 μg) was digested with *Sac*I fractionated on an agarose gel, and then analyzed by standard Southern blot hybridization procedures (Sambrook *et al.* 1989).

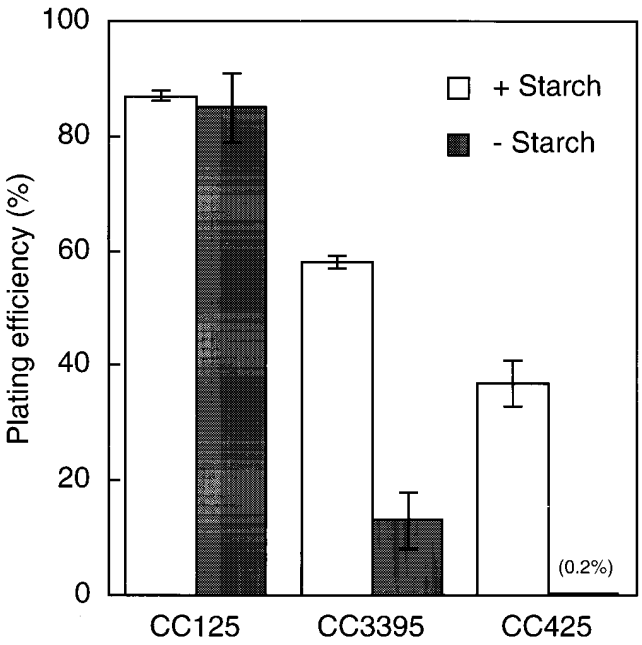

Figure 1.—Improvement of plating efficiencies by the starch embedding method. Five hundred cells of the indicated strains were plated onto solid TAP medium with or without being embedded in starch. The colonies that grew are presented as a percentage of the number of cells spread onto the medium. Error bars indicate standard deviation ($n = 2$).

RESULTS

Enhancement of plating efficiency using the starch embedding method: Cell wall-less strains of *C. reinhardtii* have considerably lower plating efficiencies than those that synthesize a wall (Harris 1989). Examination of wall-less cells spread onto the surface of solid medium revealed that most of the cells became flattened and lysed as the surface dried. The loss of moisture around the cells probably caused cell death, which could be prevented by plating the cells in an appropriate supporting medium. Many different supporting media (*e.g.*, sodium alginate, mineral oil, soft agar, various water soluble or insoluble polymers, and starches of various grains) were tested. While all were effective to some extent, the best results were obtained by embedding the cells in a suspension of corn starch. Changes in plating efficiencies for both the walled and wall-less strains as a consequence of corn starch embedding are shown in Figure 1. Without the starch, the plating efficiency of strain CC3395 was about 15% while that of CC425 was approximately 0.2%. Embedding the cells in the starch improved the plating efficiencies to 60% and 40% for CC3395 and CC425, respectively. The plating efficiency of the wild-type, cell-walled strain, CC125, was high even in the absence of the supporting medium.

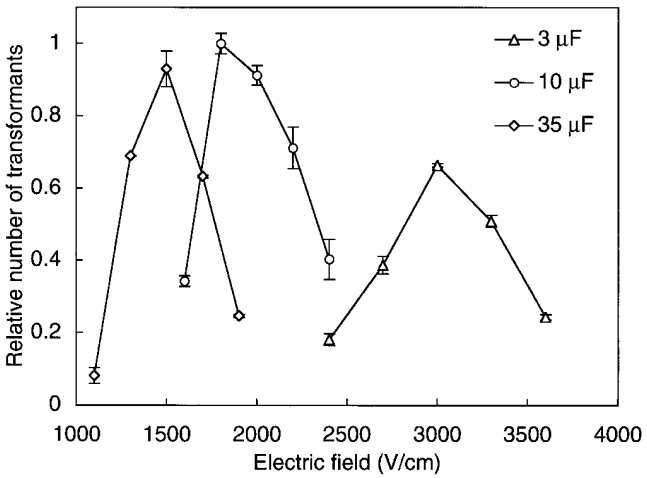

Figure 2.—Electrical field strength dependency of transformation frequency using different capacitors. *C. reinhardtii* strain CC3395 cells were electroporated in a 250-μl reaction mixture at a cell density of 1×10^8/ml with 10 μg/ml pJD67 linearized with *Hin*dIII, and 200 μg/ml of carrier DNA in TAP supplemented with 60 mm sucrose. The cells were incubated at 10° prior to the electroporation at three different capacitances (3, 10, and 35 μF). The relative transformation frequencies are plotted, with the maximum frequency set at 1. The time constants with capacitors of 3, 10, and 35 μF were 1–2, 5–6, and 15–20 msec, respectively. Error bars connect the values of duplicate samples.

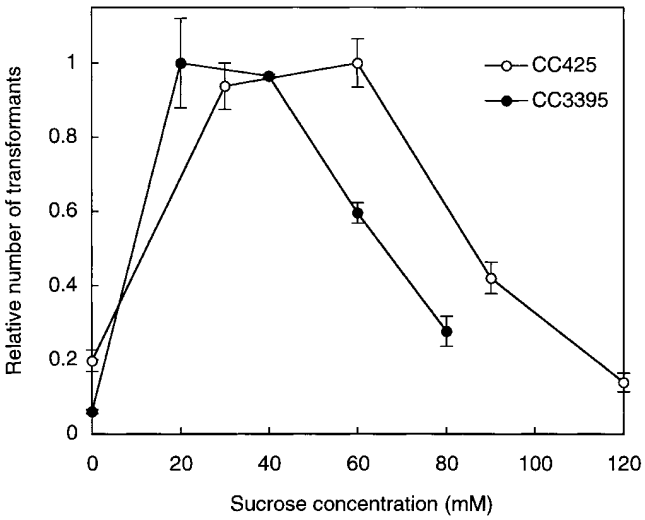

Figure 3.—Effect of osmolarity on transformation frequency. *C. reinhardtii* strains CC3395 and CC425 were electroporated using a 10 μF capacitor at 1900 V/cm and 2400 V/cm, respectively. Electroporation was in TAP medium supplemented with the indicated concentration of sucrose. Other conditions were the same as presented in the legend of Figure 2. The relative transformation frequencies are plotted, with the maximum frequency set at 1. Error bars connect the values of duplicate samples.

In the transformation experiments described below, we routinely used the starch embedding method.

Optimization of transformation efficiency: We tested five parameters that appeared to contribute to the efficiency of transformation: temperature, osmolarity, electric conditions (electric field strength and time constant of discharge) and concentration of exogenous DNA.

Figure 2 shows the electric field strength dependency of transformation of CC3395 using three different capacitances (3, 10, and 35 μF). The optimal transformation frequency was nearly identical at 10 μF and 35 μF, but was attained at two different field strengths (1500 V/cm for the 35 μF capacitor and 1800 V/cm for the 10 μF capacitor). Similar results were obtained for strain CC425; however, the electric field strength that gave optimum transformation frequencies was higher (2300–2400 V/cm with 10 μF capacitor, data not shown). We used a 10 μF capacitor in the remaining experiments.

As shown in Figure 3, the addition of sucrose to between 20 and 60 mm greatly improved the transformation frequency. Identical results were obtained when the sucrose was replaced with sorbitol (data not shown). Optimum transformation required between 20 and 40 mm sucrose for CC3395 and between 30 and 60 mm sucrose for CC425. Unless otherwise specified, the remaining experiments were performed in the presence of 40 mm sucrose, which gave near optimal results for both strains.

The effect of the concentration of heat-denatured carrier DNA on the transformation frequency is shown in Figure 4. The transformation frequency for both CC3395 and CC425 increased with increasing carrier DNA up to approximately 200 μg/ml; at concentrations

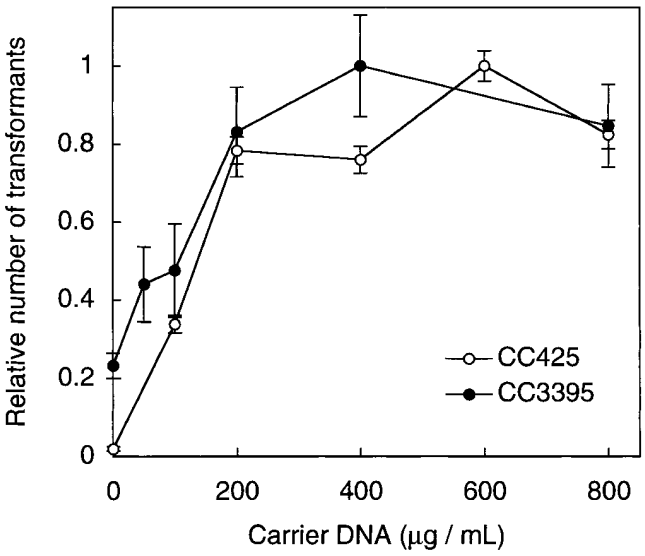

Figure 4.—Effect of carrier DNA concentration on transformation frequency. *C. reinhardtii* strains CC3395 and CC425 were electroporated with 10 μg/ml of *Hin*dIII linearized pJD67 and with indicated concentration of carrier DNA. The relative transformation frequencies are plotted, with the maximum frequency set at 1. Other conditions were the same as those described in the legend of Figure 3. Error bars connect the values of duplicate samples.

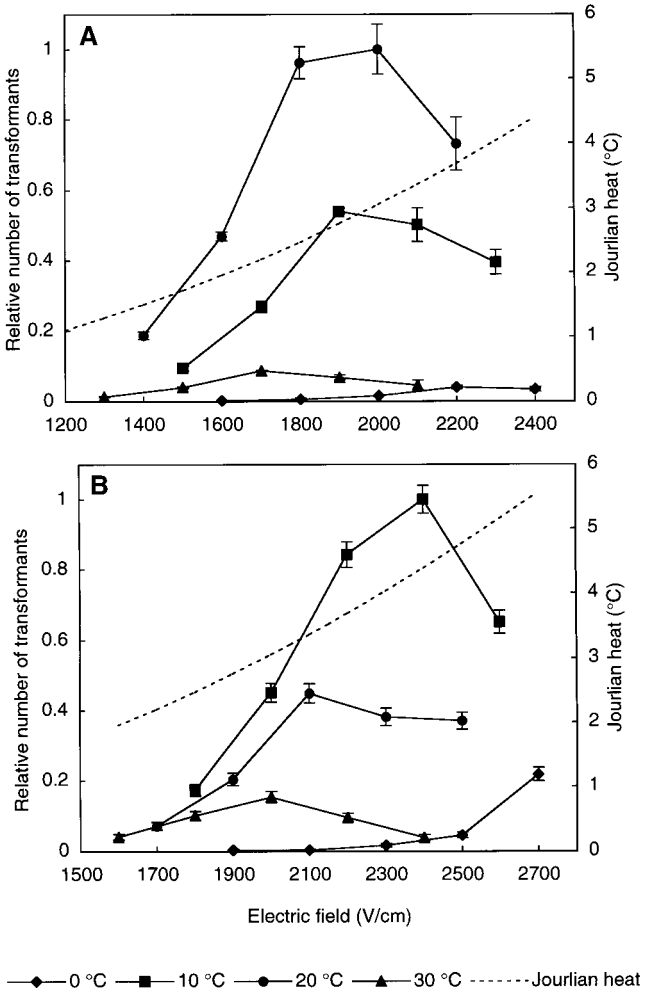

Figure 5.—Effect of temperature on transformation frequency. The electrical field strength dependency of the transformation frequency with the strain (A) CC3395 and (B) CC425 at 0, 10, 20 and 30°. The TAP medium was supplemented with 40 mm sucrose for CC3395 and 60 mm sucrose for CC425. The relative transformation frequencies are plotted, with the maximum frequency set at 1. Other conditions were the same as given in the legend of Figure 2. The broken line shows the calculated temperature increase caused by Jourlian heat generated during the electroporation. Error bars connect the values of duplicate samples.

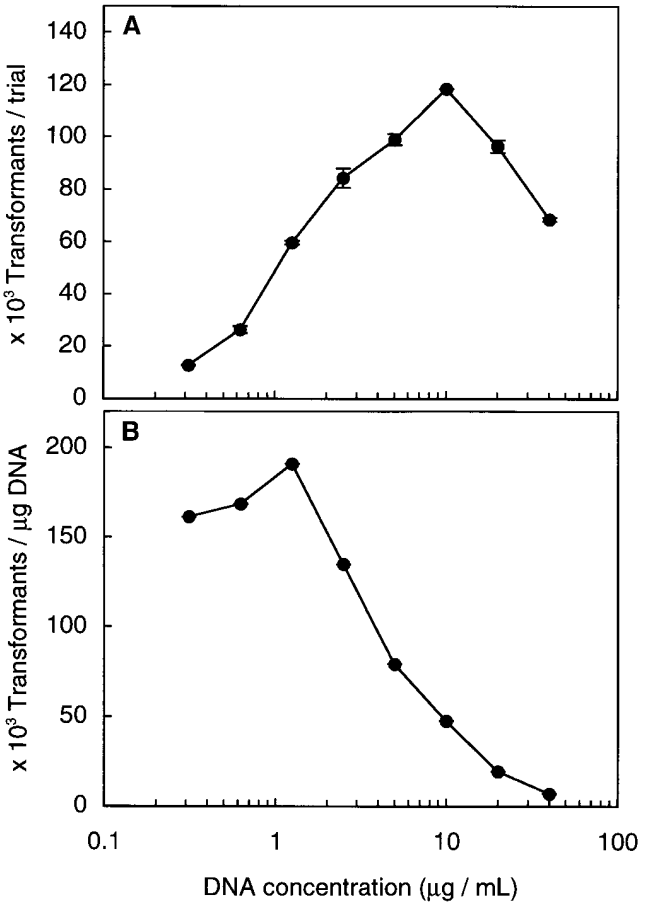

Figure 6.—Effect of DNA concentration on transformation frequency. Strain CC3395 was transformed with the indicated concentration of *Hin*dIII linearized pJD67 DNA and 200 μg/ml of carrier DNA in 40 mm sucrose-supplemented TAP. Electroporations were performed at 1800 V/cm, 20° with 10 μF capacitor and the cell density was maintained at 4×10^8/ml. (A) Transformation frequency expressed as the total number of transformants per trial (1×10^8 cells). (B) Transformation efficiency expressed as the number of transformants per μg DNA. Error bars connect the values of duplicate samples.

up to 800 μg/ml it remained nearly constant. In subsequent experiments we maintained the carrier DNA concentration at 200 μg/ml.

The temperature at which the cells were maintained prior to electroporation also had a marked effect on the transformation frequency. For CC3395, the temperature that yielded the highest transformation frequency was 20° (Figure 5A), while optimal transformation of CC425 occurred when the cells were maintained at 10° (Figure 5B). Transformation frequencies with cells kept at 0° or 30° were much lower. The rise in temperature as a consequence of the Jourlian heat generated during the electric discharge was estimated at between 3° and 4° at the optimum V/cm. It was also observed that when the incubation temperature was increased from 10° to 20°, the voltage required for optimal transformation was shifted down by roughly 100 V/cm.

Figure 6 shows the transformation frequency of CC3395 as a function of the concentration of linearized pJD67. The number of transformed cells increased gradually, reaching a maximum at a concentration of approximately 10 μg/ml (Figure 6A). At concentrations above this the frequency seemed to decrease to some extent. Normalized to the level of exogenous DNA, the maximum efficiency of transformation was at a DNA concentration of approximately 1 μg/ml (Figure 6B); at this concentration we obtained 1.9×10^5 transformants per μg DNA.

Southern blot analysis of integrated DNA: To demonstrate that the exogenous DNA integrated into the genome of the transformants, total DNA from 18 independent transformants of CC3395 was analyzed by Southern

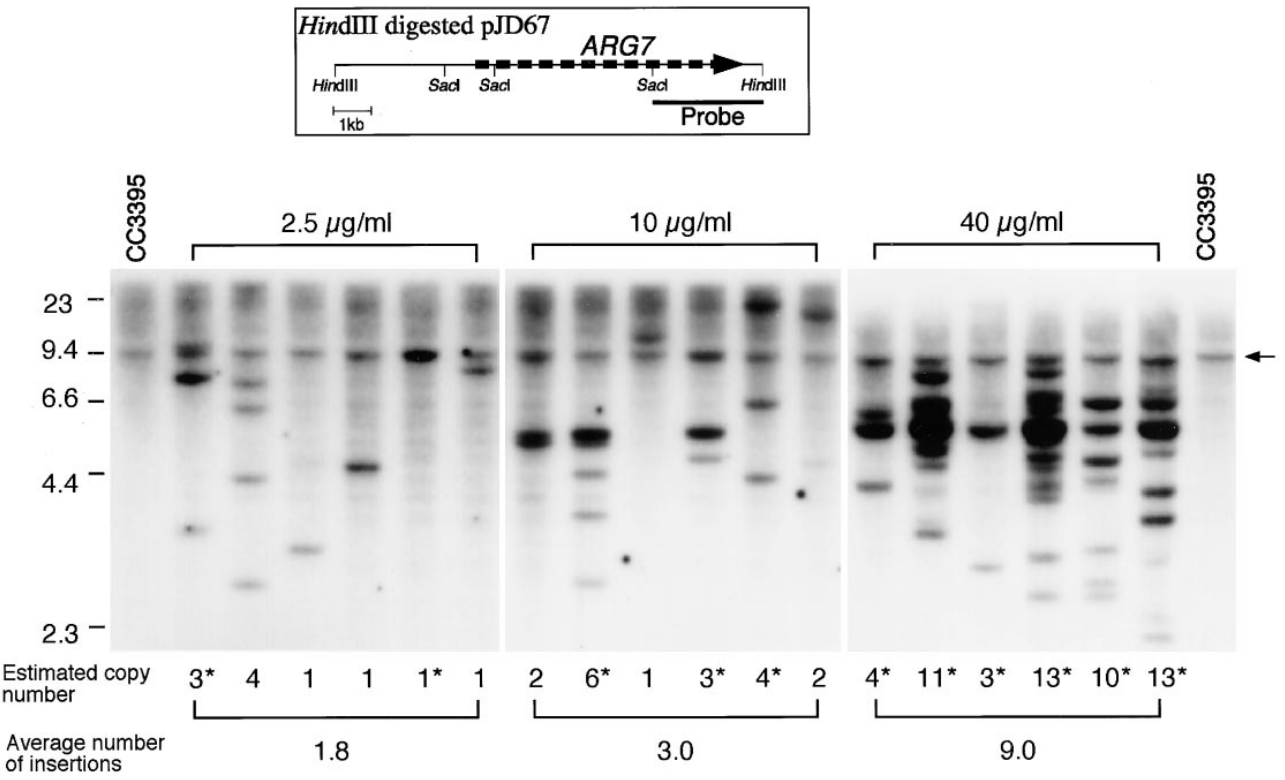

Figure 7.—Southern blot analysis of genomic DNA of the transformants. Cells were transformed in the presence of three different concentrations of *Hin*dIII linearized pJD67 DNA (2.5, 10, and 40 μg/ml, indicated at the top). Genomic DNA was prepared from individual transformants, as described in materials and methods. To visualize the introduced copies of the *ARG7* gene, a *Sac*I/*Hin*dIII fragment of 2.9 kb from pJD67 (which contains the 3′ end of the *ARG7* gene; see the inset map) was hybridized to *Sac*I digested genomic DNA from the various transformants. The right-most and left-most lanes have *Sac*I digested DNA from untransformed cells. The hybridization signal corresponding to the endogenous *ARG7* gene is indicated by an arrowhead. The average number of copies of exogenously introduced *ARG7* present in the genomes of the transformants was estimated as the number of bands that hybridized to the probe. The estimated copy number of integrated DNA for each transformant is given below each of the lanes. For the lanes having doublets, as deduced from relative band intensities, the number of hybridizing bands is appended with an asterisk (the doublets were counted as two bands).

blot hybridizations using the *ARG7*-specific probe (Figure 7). Genomic DNA from untransformed cells exhibited one hybridizing band of approximately 10 kbp, which corresponds to the intrinsic (mutated) *ARG7* gene (Figure 7, left-most and right-most lanes). All of the genomic DNA samples prepared from the transformants exhibited additional hybridizing bands. These results clearly demonstrated that the genomes of the transformants carried additional copies of the *ARG7* gene. The copy number of the integrated DNA correlated with the concentration of the exogenous pJD67 DNA used during the electroporation; transformations with 2.5, 10 and 40 μg/ml of the exogenous DNA resulted in an average of 1.8, 3.0 and 9.0 copies of the *ARG7* gene per genome (Figure 7).

Final conditions and frequencies of transformation: The final conditions used to obtain maximum transformation frequencies are summarized in Table 1. The final scores obtained with CC3395 and CC425 under the optimized condition are given in Table 2. Electroporation of 2.5×10^7 cells (1.0×10^8/ml) resulted in 6.6×10^4 transformants for strain CC3395; the transformation frequency was 2.6×10^{-3}. When the cell density was increased by 4-fold (4.0×10^8/ml), the number of total transformants increased by 3.2-fold (2.1×10^5/trial), and the transformation frequency decreased slightly (2.1×10^{-3}). At the higher cell density (4.0×10^8/ml), the number of transformants obtained with CC425 was

TABLE 1

Standard conditions for electroporation of DNA into *Chlamydomonas reinhardtii*

Sample conditions	
Sample size	250 μl in 4-mm cuvette
Cell number	$0.25–1 \times 10^8$ cells ($1–4 \times 10^8$/ml)
Exogenous DNA	2.5 μg (10 μg/ml)
Carrier DNA	50 μg (200 μg/ml)
Transformation medium	TAP + 40 mm sucrose
Temperature	10–20°
Electric conditions	
Electric field strength	1800–2300 V/cm
Time constant	5–6 msec (10 μF capacitor)

TABLE 2

Comparison of transformation efficiencies with electroporation and glass bead method

A. Electroporation

Strain and genotype	Exogenous DNA[a]	Electric field strength (V/cm)	Temp.	No. of cells per trial ($\times 10^8$)	Starch embedding	Transformants per trial[b] ($\times 10^3$)
CC3395 *arg7-8 cwd*	pJD67	1900	20°	1.0	+	210 ± 50
				0.25	+	66 ± 11
				0.25	−	18 ± 4
CC3395 *arg7-8 cwd*	42G2	1900	10°	1.0	+	24 ± 2
CC425 *arg7-8 cw15*	pJD67	2300	10°	1.0	+	136 ± 20
				1.0	−	7 ± 6

B. Glass bead transformation[c]

Strain and genotype	Exogenous DNA	Time of vortexing (sec)	No. of cells per trial ($\times 10^8$)	Starch embedding	Transformants per trial ($\times 10^3$)
CC3395 *arg7-8 cwd*	pJD67	20	0.25	−	0.11 ± 0.04
CC425 *arg7-8 cw15*	pJD67	20	1.0	+	0.88 ± 0.07
			1.0	−	0.82 ± 0.06

[a] 2.5 μg per assay.
[b] Mean ± SD (n = 3).
[c] Performed as in Kindle (1990).

slightly less (1.4×10^5/trial) than that for CC3395. For both strains the transformation frequencies declined if the cells were plated without embedding them in starch (under optimal conditions the number of transformants was reduced to 1/4 and 1/20 of the maximal levels for CC3395 and CC425, respectively). When the cosmid clone containing the *ARG7* gene (clone 42G2) was used as the exogenous DNA instead of pJD67, the transformation frequencies declined by approximately one order of magnitude (2.4×10^4 transformants per trial, Table 2). The results also clearly demonstrate that the frequency of transformation using electroporation was much higher (approximately two orders of magnitude) than that obtained by the glass bead procedure (compare Table 2A and 2B).

DISCUSSION

We report here detailed conditions for optimal transformation by electroporation of *C. reinhardtii* strains CC3395 and CC425. The final number of transformants that we obtained reflects both an increase in the survival of the cells during plating in a supporting medium plus an increase in the efficiency of introducing the exogenous DNA into the cells. While several other attempts have been made to achieve high frequency transformation of *C. reinhardtii* using electroporation (Brown *et al.* 1991; Butanaev 1994), the efficiency achieved in this study was far better than those reported previously. Recently, Tang *et al.* (1995) reported a procedure in which they were able to successfully electroporate DNA into *C. reinhardtii*. While relatively high numbers of transformants were obtained (500 transformants from 1.8×10^5 cells using 2 μg exogenous DNA), the transformation efficiency (250 transformants per μg DNA) was much lower than reported in this article (1.9×10^5 transformants per μg DNA; Figure 6B). Furthermore, Tang *et al.* (1995) used specialized equipment for imposing the electric field.

The absolute transformation frequency that we observed was between 1.5- and 2-fold higher for CC3395 than for CC425, and the conditions required for optimal transformation were also somewhat different between the strains. Variations in transformation characteristics among strains may reflect differences in the average diameter of the cells, the rigidity and composition of the cytoplasmic membranes and the metabolism of the cells. Metabolic variation between CC3395 and CC425 is reflected in the different growth rates of the two strains; it takes 3 days for CC3395 to form detectable colonies on solid medium while colonies of CC425 are observed only after a week.

Starch embedding of the cells prior to spreading them onto solid medium resulted in a great increase in the final number of transformants for both CC425 and CC3395 following electroporation (Table 2). This reflects improved plating efficiency (viability) on the solid medium (Figure 1). Interestingly, the starch embedding procedure did not result in increased numbers of transformants if the DNA was introduced by the glass bead

method (Table 2). For the glass bead method, the entire transformation mixture is spread on a single plate because of the lower transformation frequency. Therefore the density of cells placed on the plate following glass bead transformation is far greater than that used following electroporation. The high density of untransformed cells may act as a protective medium similar to the starch granule embedding medium.

To attain the highest transformation frequency, a number of different parameters were critical; these included electric conditions (electric field strength and time constant), temperature and osmolarity. High efficiencies of transformation occurred over a narrow range of temperatures (10–20°) and osmolarities (30–60 mm of added sucrose). The reason for the pronounced temperature effect is not clear, although decreasing the temperature would make the membranes more rigid, which in turn might make it more difficult for the cells to reseal following the electric pulse. The final number of transformants would be determined by the facility with which the DNA enters the cell and integrates into the genome, and the number of cells that die because of irreversible damage caused by disruption of the cell membrane; the temperature might affect the balance between these processes at any given voltage. The addition of an appropriate osmoticum to the electroporation medium may lead to an increase in the number of viable transformants by minimizing cell lysis that results from the electric pulse. This is supported by microscopic observation of the cells after electroporation; in the absence of sucrose, most of the cells appeared to be lysed immediately following the electric pulse, while little lysis was observed if electroporation was performed on cells in TAP medium containing 40 mm sucrose. Microscopic observation also revealed that pulsation of contractile vacuoles, by which permeated water is pumped out of the cell, stopped when the cells were suspended in the sucrose-enriched TAP, which indicates that the sucrose makes the medium nearly isotonic with the cytoplasm of the cell.

The concentration of exogenous DNA that resulted in the maximum number of transformants per trial at a given cell density was 10 μg/ml. However, the concentration of DNA that led to the maximum number of transformants per μg of DNA was approximately 1 μg/ml (Figure 6B). The concentration of DNA also affected the number of copies of the exogenous DNA that inserted into the nuclear genome (Figure 7). Insertion of more than one copy of DNA is not desirable for the purpose of insertional mutagenesis; it would make the identification of tagged genes more difficult. Therefore, for generating tagged mutants the exogenous DNA should be maintained at a concentration that yields approximately one integration event per transformant. When multiple copies of the inserted DNA are not a great disadvantage (*e.g.*, complementation of mutants), one could use a relatively high concentrations of exogenous DNA.

Another factor that contributed to the improvement of the final transformation frequency was the addition of carrier DNA. With the glass bead method it was reported that the addition of carrier DNA (50 μg/assay) increased the transformation frequency moderately (1.8-fold). However, when carrier DNA was included in the electroporation mixture the transformation frequency increased by approximately one order of magnitude (Figure 4). A large enhancement of the transformation frequency by the addition of single-stranded DNA was also reported for yeast (Schiestl and Gietz 1989). Little is known about how the carrier DNA elevates the frequency of transformation. It may act as a competitive inhibitor of nucleases, thereby protecting the exogenous DNA from degradation and/or block nonspecific DNA binding to the cell surface. However, if the carrier DNA integrates into the genome of transformed cells, it may cause difficulties in experiments in which an attempt is being made to create mutants and tag the altered genes by a specific DNA sequence such as that of ARG7 (Davies *et al.* 1996). There are a number of ways in which this problem can be resolved. It is possible to omit the carrier DNA from the electroporation reaction mixture and still maintain a relatively high transformation frequency. Furthermore, rRNA can serve the same purpose as carrier DNA (Schiestl and Gietz 1989) and would not be able to integrate into the genome.

The frequency with which we have been able to introduce DNA into *C. reinhardtii* by electroporation is approaching that obtained for yeast (for yeast, 1 μg of DNA can yield as many as 10^6 transformants) (Manivasakam and Schiestl 1993; Gietz *et al.* 1995). This high frequency of transformation is critical when trying to complement mutants of *C. reinhardtii* with recombinant phage or cosmid libraries. In *C. reinhardtii*, the transformation efficiency with cosmid DNA was about one order of magnitude lower than that obtained with the plasmid DNA (Table 2, 42G2 compared to pJD67), which is probably a consequence of the difference in the sizes of the DNA molecules. A similar dependency of transformation frequency on DNA molecule size was reported for *E. coli* (Hanahan 1983).

The complementation of mutants of *C. reinhardtii* with a cosmid library has become feasible given the frequency of transformation attained in these studies. Transformation with the cosmid 42G2 yielded approximately 2.4×10^4 transformants per trial (Table 2). Recombinant cosmid clones of 1×10^4 would have a more than 90% chance (Zhang *et al.* 1994) of containing any particular *C. reinhardtii* gene. Therefore, with a single electroporation trial using the cosmid library, we are likely to be able to complement any recessive mutation. Indeed, we have recently used the high efficiency transformation procedure reported here to complement a mutant that does not properly acclimate to phosphorus starvation (D. D.

Wykoff, D. P. Weeks, J. L. Kovar, H. Usuda, A. R. Grossman and K. Shimogawara, unpublished results). Furthermore, the increased transformation frequency would potentially make targeted disruptions of genes easier. With *C. reinhardtii* it was reported that the ratio of homologous to nonhomologous recombination events was ~1/1000 with the glass bead method or ~1/24 with the microprojectile bombardment method (Sodeinde and Kindle 1993). Although we have not examined the frequency of homologous recombination with electroporation, we should be able to isolate several homologous recombinants with a single electroporation trial. Finally, the increased level of transformation would also help facilitate the use of reporter genes (Davies *et al.* 1992) for evaluation of expression from regulated promoters (Davies and Grossman 1994).

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