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Genetics

High-frequency nuclear transformation of *Chlamydomonas reinhardtii*

(algae/glass beads/transfection)

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ABSTRACT **By using a method in which cell-wall-deficient *Chlamydomonas reinhardtii* cells were agitated in the presence of DNA, glass beads, and polyethylene glycol, nuclear transformation rates of $\approx 10^3$ transformants per μg of plasmid DNA were achieved. The nitrate reductase gene from wild-type *Chlamydomonas* was used to complement a mutation in the corresponding gene of a strain containing *nit1-305*. Transformants were selected by growth with nitrate as sole source of nitrogen. The transforming DNA integrated into the genome at a low-copy number in nit$^+$ transformants. When cells carrying *nit1-305* were agitated in the presence of two plasmids, one with the gene for nitrate reductase and the second with an unselected gene, the unselected gene was present in 10–50% of nit$^+$ transformants. This high frequency of cotransformation will allow any cloned gene to be introduced into *Chlamydomonas*. Moreover, the overall efficiency of transformation should be high enough to permit isolation of genes from genomic libraries by complementation of stable nuclear mutants. The availability of efficient nuclear and chloroplast transformation in *Chlamydomonas* provides specific advantages for the study of chloroplast biogenesis, photosynthesis, and nuclear–chloroplast genome interactions.**

The ability to genetically transform an organism by introducing DNA into its nuclear and/or organellar genomes greatly enhances the utility of that organism for experimental research. *Chlamydomonas reinhardtii* is a single-celled eukaryotic alga that has been extensively used for studies of flagellar structure and assembly (1, 2), cell–cell interactions during mating (3), cell cycle, and photosynthesis (4, 5). Transformation of the *Chlamydomonas* chloroplast genome using DNA-coated microprojectiles accelerated by a particle gun has recently been reported (6, 7). Subsequently, low-frequency transformation of the nuclear genome was achieved with the same method (8–10, S. P. Mayfield and K.L.K., unpublished work). Three *Chlamydomonas* genes have been used as selectable markers for nuclear transformation, and in all cases, the DNA complements a mutation in the corresponding structural gene. These *Chlamydomonas* genes encode nitrate reductase (allows growth on nitrate as sole nitrogen source) (8, 11), arginosuccinate lyase (corrects an arginine auxotrophy) (9, 10), and a protein in the oxygen-evolving complex of photosystem II (allows phototrophic growth) (S. P. Mayfield and K.L.K., unpublished work). Although bombardment using the particle gun is simple and reproducible, it is difficult to generate large numbers of nuclear transformants. Moreover, the apparatus is expensive and not widely available at the present time. I show below that when cell-wall-deficient *Chlamydomonas* cells are agitated in the presence of glass beads, DNA, and polyethylene glycol (PEG), transformation of the nuclear genome occurs at a high rate. This procedure, inspired by that of Costanzo and Fox for transforming yeast nuclei (12), also results in high rates of cotransformation, so that any gene can be introduced into *Chlamydomonas* cells, even if there is no observable change in phenotype.

MATERIALS AND METHODS

Strains and Plasmid DNAs. Plasmid pMN24 contains a 14.5-kilobase (kb) *Chlamydomonas* genomic DNA fragment encoding nitrate reductase (11). pRSP3-EB contains a 6.5-kb *Eco*RI–*Bam*HI fragment with the *Chlamydomonas* gene for radial spoke protein 3 (13). The cabλ3 plasmid contains a *Chlamydomonas* genomic DNA fragment encoding one of the chlorophyll a/b binding protein proteins; a 500-base-pair (bp) fragment of bacteriophage λ DNA has been inserted into the 3′ noncoding region of the gene (8).

nit1-305 is a stable NR mutation (reversion $< 10^{-8}$), originally isolated in wild-type strain 6145c (14, 15). A cell-wall-deficient strain (*cw-15*) (16) carrying this mutation was constructed by P. Lefebvre (University of Minnesota) and contributed for this study. H3 strain was constructed by P. Lefebvre by crossing the *nit1-305*-carrying mutant with a paralyzed flagellar (*pf-14*) mutant. The paralyzed flagellar phenotype is due to a missense mutation in radial spoke protein 3 (13, 17). High-efficiency-mating wild-type strains CC620 and CC621, used for preparation of gamete autolysin, were obtained from the Chlamydomonas Genetics Center, Duke University.

Cell Culture and Transformation Protocol. Sager–Granick medium containing ammonium (SGII-NH_4 medium) (18) was made by adding the following to 1 liter of distilled water: 3.67 g of NaH_2PO_4, 1.15 g of K_2HPO_4, and 2 g of sodium acetate (anhydrous); in addition 10 ml of each of the following stock solutions was added: 0.1% $FeCl_3$, 0.4% $CaCl_2 \cdot 2\ H_2O$, 1.5% (wt/vol) $MgSO_4$, 3% (wt/vol) NH_4NO_3, and trace elements (trace element stock solution contained 100 mg of H_3BO_3, 100 mg of $ZnSO_4 \cdot 7\ H_2O$, 40 mg of $MnSO_4 \cdot 4\ H_2O$, 20 mg of $CoCl_2 \cdot 6\ H_2O$, 20 mg of $Na_2MoO_4 \cdot 2\ H_2O$, and 4 mg of $CuSO_4$ in 1 liter). In Sager–Granick medium lacking ammonium (SGII-NO_3 medium), KNO_3 replaced NH_4NO_3. The 1/2R medium was similar, except it contained twice as much $MgSO_4$, the solid NaH_2PO_4 and K_2HPO_4 were omitted, and 2.2 ml of 10% (wt/vol) KH_2PO_4 was added. The pH of 1/2R medium was adjusted to 7.0 with 10% K_2HPO_4.

Mutant stocks were maintained on YA (yeast extract-acetate) plates (19). *nit1-305* mutants were inoculated from plates into 1/2R liquid medium and grown to a concentration of $\approx 3 \times 10^6$ cells per ml and then diluted into SGII-NH_4 medium. Cells were grown to $1\text{–}2 \times 10^6$ cells per ml and then harvested by centrifugation at $5000 \times g$ and resuspended in

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Abbreviations: SGII-NH_4 and SGII-NO_3, Sager–Granick medium II containing or lacking ammonium, respectively; PEG, polyethylene glycol.

SGII-NO_3-selective liquid medium at a concentration of $\approx 10^8$ cells per ml.

PEG (M_r 6000) from Sigma (P-5413) was made as a 20% (wt/vol) stock solution and added to cells, usually at a final concentration of 5% (wt/vol), immediately before transformation.

Autolysin was prepared from mating CC620 and CC621 cells as described (19), except that the dialysis step was omitted and the crude autolysin was filtered through a 0.2-μm Uniflow filter (Schleicher & Schuell) and frozen before use to kill any remaining cells. Cell walls were removed from *nit1-305 cw*$^+$ cells by incubating them in undiluted autolysin for 30–60 min at room temperature; effectiveness of treatment was monitored by sensitivity to 0.004% Nonidet P-40 detergent (Sigma). Cells were harvested from autolysin by centrifugation, resuspended in selective liquid medium, and transformed immediately to avoid cell-wall regeneration.

Glass beads, 0.45–0.52 mm in diameter, were obtained from Thomas. They were washed with concentrated sulfuric acid, then rinsed thoroughly with distilled water, dried, and sterilized by baking at 250°C for 2–3 hr. Glass beads (300 mg) were added to 0.4 ml of cells, DNA was added, and cells were agitated at top speed on a Fisher Vortex Genie II mixer in 15-ml conical disposable polypropylene centrifuge tubes (Corning). The beads were allowed to settle, and cells were spread on selective agar plates with a glass spreader. Selection for *nit1* transformants was done on SGII plates in which KNO_3 replaced NH_4NO_3 (SGII-NO_3); in addition, the agar was washed with distilled water to remove ammonium ions. The plates were allowed to dry and then sealed with Parafilm and incubated in a 25°C growth chamber under a 14-hr light/10-hr dark illumination schedule. *nit*$^+$ transformant colonies were visible after 6–8 days of growth.

Preparation and Analysis of DNA. Plasmid DNA was prepared by the modification of an alkaline extraction procedure (20). *Chlamydomonas* genomic DNA was purified from 100-ml liquid cultures, and DNA blot analysis was performed as described (8). Autoradiographic bands were quantified on a Bio-Rad model 620 video-densitometer.

RESULTS

Recovery of Nuclear Transformants by Agitating Cells with DNA. A cell-wall-deficient strain (*cw-15*) (16) carrying a mutation in the structural gene for nitrate reductase (*nit1-305*) was used as the recipient for transformation experiments. *nit1-305 cw-15* cells were grown in nonselective liquid medium (SGII-NH_4), harvested, and resuspended in selective liquid medium (SGII-NO_3). The cells were then agitated in the presence of 0.5-mm glass beads and DNA from plasmid pMN24, which carries the structural gene for nitrate reductase (11). After agitation, cells were immediately plated on solid medium with nitrate as the sole source of nitrogen (SGII-NO_3) to select colonies with functional nitrate reductase. After 1 week, nit$^+$ colonies appeared in samples that had been agitated in the presence of DNA. Fig. 1 shows that the optimal time of agitation for recovery of nit$^+$ transformants was between 10 and 20 sec. Both linearized and supercoiled DNA gave rise to nit$^+$ colonies, although linearized DNA was somewhat more efficient. Cell viability declined during agitation; after 60 sec only $\approx$25% of the cells remained viable (data not shown). Although *Chlamydomonas* cells survived agitation in the presence of glass beads for longer periods than do yeast cells (12), the optimal time for recovery of transformants was shorter.

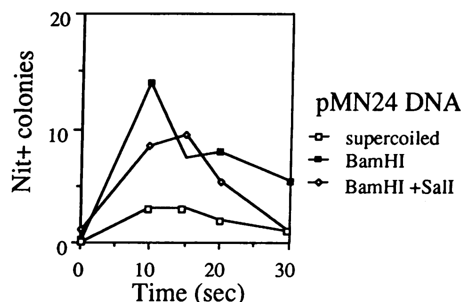

FIG. 1. nit$^+$ colonies generated by agitating cells with glass beads for different agitation periods. *nit1-305 cw-15* cells were transformed to nit$^+$ by agitation with plasmid pMN24 and beads as described; pMN24 DNA was either supercoiled, linearized by digestion with *Bam*HI, or cleaved with *Bam*HI plus *Sal* I to generate a 10-kb fragment containing the nitrate reductase gene.

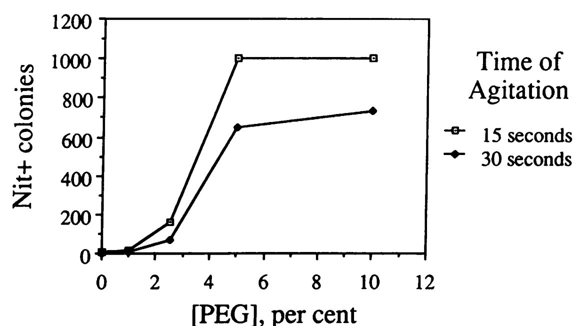

FIG. 2. Effect of PEG on transformation efficiency. *nit1-305 cw-15* cells were grown to 1.5×10^6 cells per ml, harvested, and resuspended 1:100 in SGII-NO_3 liquid medium. H_2O (0.1 ml) or 0.1 ml of PEG/H_2O was added to 0.3 ml of cells and 2 μg of pMN24 DNA digested with *Bam*HI plus *Sal* I. Glass beads were added, and cells were agitated and immediately spread on selective SGII-NO_3 agar plates as described.

A number of parameters were tested for their effect on transformation. The most dramatic increase was achieved by agitating cells with PEG. Fig. 2 shows that 5% PEG caused a 100-fold increase in the number of transformants recovered in this experiment. Because 10% PEG did not further stimulate transformation, subsequent experiments were done with 5% PEG. Table 1 shows the effects of other modifications of the procedure on transformation rates. Spermidine (30 μM) and hexamine cobalt (40 μM), which cause DNA to condense under some conditions (21), did not affect transformation rates, whereas single-stranded carrier DNA (50 μg) and dimethyl sulfoxide (1%) increased transformation modestly. A slight increase in transformants was achieved when cells were plated in soft agar rather than being spread on the surface of agar plates. The addition of 0.2 M mannitol (for

Table 1. Effect of various treatments on transformation rate

Treatment	nit$^+$ colonies
Standard protocol	200
+ hexamine cobalt(III) (40 μM)	216
+ spermidine (30 μM)	225
+ carrier DNA (50 μg)*	352
+ 0.2 M mannitol	15
+ 1% dimethyl sulfoxide	320
Plated in soft agar†	424

nit1-305 cw-15 cells were grown to 1.5×10^6 cells per ml and transformed in the presence of 5% PEG as described, except for the noted additions to this standard protocol. In this experiment, 5×10^7 cells were transformed with 2 μg of DNA, and one-fifth of the cells were plated on SGII-NO_3 plates.

*Salmon sperm DNA (Sigma) was sheared by sonication and denatured by heating at 100°C for 10 min.

†Cells were plated in soft agar by adding 80 μl of transformed cells to 3 ml of 0.7% agar in SGII-NO_3 at 45°C and pouring on top of a 100-mm SGII-NO_3 agar plate.

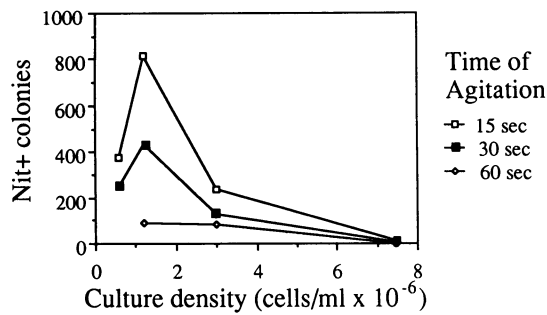

FIG. 3. Effect of growth phase on recovery of nit^+ transformants. *nit1-305 cw-15* cells were grown in $SGII-NH_4$ medium and harvested after various times of culture for transformation. Cells were resuspended in $SGII-NO_3$ liquid medium at 3×10^8 cells per ml. Each sample contained 0.3 ml of cells, 300 mg of glass beads, 5 μg of pMN24 DNA digested by *Bam*HI and *Sal* I, and 0.1 ml of 20% PEG. In controls in which DNA was added but the cells were not agitated there were no nit^+ colonies (data not shown).

osmotic pressure) had a deleterious effect on transformant recovery, although mannitol is not toxic to cells at this concentration (data not shown). *Chlamydomonas* cells appear to be transformed more efficiently when harvested early in culture (Fig. 3), although transformants have been generated even from stationary cultures. The number of transformants that were recovered did not appear to depend on the DNA concentration, at least in the range of 2.5–50 μg/ml (1–20 μg per transformation) (Fig. 4). The size of the glass beads did not seem critical; transformants have been generated using 0.1-mm beads, glass wool, and even by agitating cells without any particles (data not shown). The protocol has been optimized for 0.5-mm glass beads. That mild shear allows *Chlamydomonas* cells to take up DNA suggests that this process could occur in nature.

Initial transformation experiments with a *nit1-305* strain containing an intact cell wall rarely yielded transformants. When such cells were treated with autolysin, an enzyme normally released by mating *Chlamydomonas* cells to induce cell-wall removal (19, 22), they were transformed at rates similar to cell-wall-deficient (*cw-15*) derivatives of *nit1-305* (Table 2). The rate of transformation was further enhanced by adding 5% PEG.

Plasmid pMN24 DNA in Genomic DNA of nit^+ Transformants. Genomic DNA was prepared from transformants that had been obtained by agitating cells with various amounts of DNA. The DNA was digested with *Pvu* II, blotted to nylon filters, and hybridized with labeled pMN24 DNA. Genomic DNA from the untransformed *nit1-305*-containing recipient contained three *Pvu* II fragments that hybridized to the probe

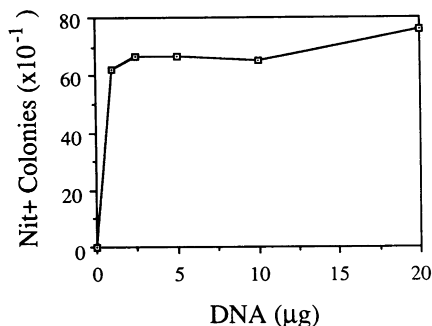

FIG. 4. Effect of increased amounts of DNA on recovery of nit^+ transformants. *nit1-305 cw-15* cells were grown to 3×10^6 cells per ml, harvested, and transformed in the presence of 5% PEG as described, except that added DNA was varied between 0–20 μg per transformation (0–50 μg/ml). One tenth of the cells was diluted and plated on $SGII-NO_3$ plates.

Table 2. Effect of PEG and autolysin on transformation of cw^+ cells

Autolysin	5% PEG	Time, sec	nit^+ colonies
–	–	0	0
–	–	15	0
–	–	30	0
–	–	60	0
–	+	0	0
–	+	15	0
–	+	30	1
–	+	60	1
+	–	0	1
+	–	15	81
+	–	30	25
+	–	60	2
+	+	0	5
+	+	15	407
+	+	30	321
+	+	60	78

nit1-305 cells were grown in $SGII-NH_4$ medium to 1.5×10^6 cells per ml and harvested for transformation as described. Half the cells were resuspended in 5 ml of autolysin and incubated for 45 min at room temperature. For transformation, 0.3 ml (5×10^7 cells) with or without autolysin treatment was added to 0.1 ml of H_2O or 20% PEG in H_2O and agitated in the presence of 2 μg of *Bam*HI-digested pMN24 DNA as described for Fig. 2.

(bands A–C in Fig. 5). Most transformants also contained fragments that comigrated with plasmid pMN24 fragments (bands D and E in Fig. 5). In addition, all nit^+ colonies contained two to four extra *Pvu* II fragments hybridizing to

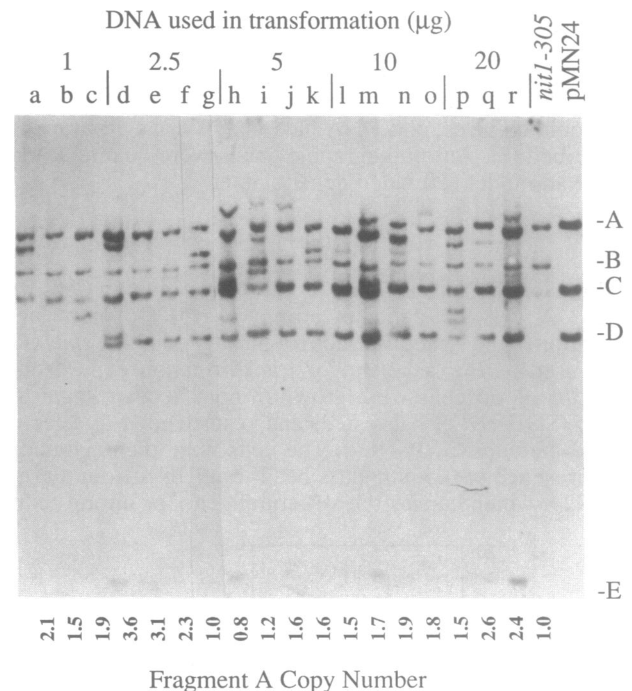

FIG. 5. pMN24 DNA sequences in genomic DNA of nit^+ transformants. *nit1-305 cw-15* cells were transformed with various amounts of *Bam*HI-digested pMN24, as described for Fig. 1. nit^+ colonies were grown in nonselective liquid medium ($SGII-NH_4$), and genomic DNA was prepared by using a miniprep procedure (8). Three micrograms of DNA was digested with *Pvu* II, fractionated on an agarose gel, blotted to Nytran (Schleicher & Schuell), and hybridized to a radioactive probe for pMN24. The copy number of fragment A, which is present both in genomic DNA and in the transforming plasmid pMN24, was determined by densitometry. Variations in loading were corrected by normalizing to the intensity of fragment B, which is present in genomic DNA, but not in pMN24.

the pMN24 DNA probe, which were not present either in genomic DNA from untransformed cells or in plasmid pMN24. These probably represent junction fragments between *Chlamydomonas* DNA and plasmid DNA integrated into the genome. These results suggest that one or two integration events occurred in these nit$^+$ transformants. We have previously shown that nit$^+$ transformants generated by bombardment with the particle gun contained 1–20 integrated copies of pMN24 DNA, which were inherited as stable Mendelian traits through meiosis (8). The copy number of pMN24-specific DNA fragments was significantly lower in transformants generated by this agitation method. The particle gun probably delivers more DNA per cell than does agitation in the presence of DNA. However, increasing the amount of DNA present during agitation did not significantly increase the amount of integrated plasmid DNA (Fig. 5).

In a few transformants (e.g., transformants e and q in Fig. 5), genomic DNA fragments that comigrated with pMN24 fragments (e.g., fragment A) appeared to be present in a higher copy number than expected from the number of junction fragments, suggesting that in these cases the pMN24 had integrated in a few tandem copies. Alternatively, there could have been a significant amount of extrachromosomal plasmid DNA in these transformants. When undigested genomic DNA from transformants was size-fractionated on an agarose gel, blotted to a nylon membrane, and hybridized with pMN24 DNA, there was a small amount of hybridization to a fragment that comigrated with undigested pMN24 DNA in about half the samples (data not shown). Although this hybridization represented a very small fraction of the hybridization to chromosomal DNA, the existence of unintegrated pMN24 DNA suggests that low-efficiency replication of the plasmid occurred. Although the plasmid DNA used for transformation had been linearized at the *Bam*HI site, it may have recircularized inside the cell. In a few transformants (e.g., transformants g and h in Fig. 5), the copy number of fragment A did not increase relative to the untransformed *nit1-305*-carrying strain. Because pMN24 had been linearized at the *Bam*HI site within fragment A, the integration event probably occurred at this site in these transformants.

Cotransformation of a Second Unselected Gene. To test whether two genes on independent plasmids could be cotransformed into *Chlamydomonas* by using this procedure, the following experiment was done. A gene for one of the chlorophyll a/b (cab) binding proteins of *Chlamydomonas* was used as the unselected DNA in cotransformation experiments using *nit1* (pMN24) as the selectable marker. The introduced chlorophyll a/b gene was marked by inserting a piece of bacteriophage λ DNA into the 3′ noncoding part of the gene. nit$^+$ colonies were selected from cells transformed with pMN24 and cabλ3 DNA, and genomic DNA was prepared from 18 such colonies and probed for the presence of the unselected DNA. In two transformants, there was a *Pst* I fragment derived from cabλ3 DNA that was not seen in untransformed genomic DNA (Fig. 6), demonstrating that cotransformation had occurred in slightly >10% of nit$^+$ colonies.

An even higher rate of cotransformation occurred in the following experiment. A cell-wall-containing strain that carries both the *nit1-305* mutation and the paralyzed flagellar mutation *pf-14* was constructed by P. Lefebvre (called H3). Diener and collaborators had shown that when the particle gun bombarded this strain with the *nit1* gene of pMN24 and the DNA for the radial spoke protein 3, pRSP3-EB (13), a very high fraction of cells transformed to nit$^+$ also recovered motility (D. R. Diener, A. M. Curry, K. A. Johnson, B. D. Williams, P. A. Lefebvre, K.L.K., and J. L. Rosenbaum, unpublished work). Table 3 shows the results of an experiment in which cells from this strain were agitated in the presence of pMN24 and pRSP3-EB DNA. The number of nit$^+$

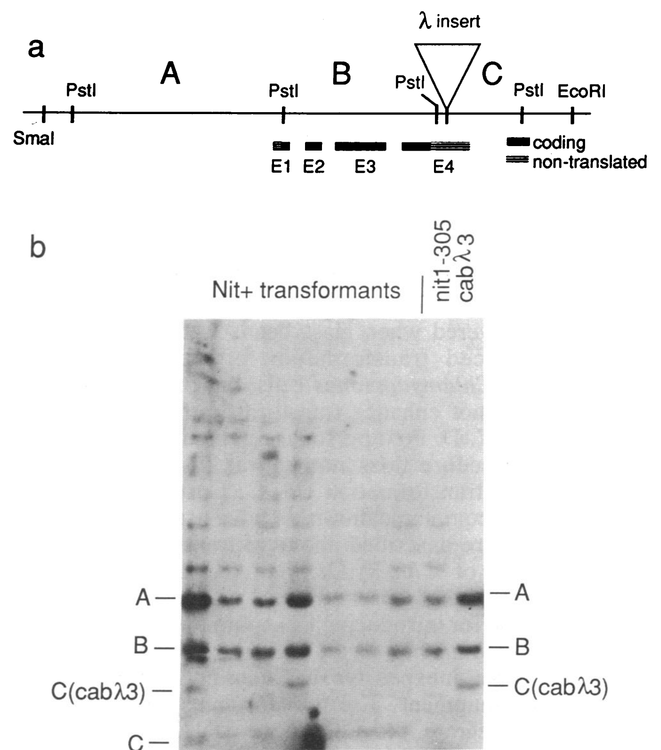

FIG. 6. cabλ3-specific sequences in some cotransformed nit$^+$ colonies. Genomic DNA was prepared from nit$^+$ colonies arising from cells that had been agitated in the presence of both cabλ3 and pMN24 DNA. After digestion with *Pst* I, cabλ3-specific fragments were visualized by hybridizing blots to a probe derived from the *Sma* I–*Eco*RI fragment, which contains the entire DNA for cabλ3. (*a*) Map of the DNA for cabλ3, showing the site of insertion of the bacteriophage λ DNA and location of the probe. E, exon. (*b*) Hybridization to genomic DNA from nit$^+$ transformants, untransformed *nit1-305* strain, and cabλ3 plasmid DNA.

transformants recovered with cell-wall-containing cells was low, ranging from three to five transformants per plate. However, the fraction of nit$^+$ transformants that had recovered motility was fairly high, ranging from 25–50%. When cells were treated with autolysin for 1 hr, the number of transformants increased substantially, and from 40–60% of the cells had recovered motility and presumably were cotransformed.

Table 3. Cotransformation with a radial spoke protein gene

pMN24 DNA, μg	pRSP3-EB DNA, μg	Autolysin	nit$^+$ colonies, no.	Motile nit$^+$ colonies, no./total scored	Motile, %
5	0	−	11	0/11	0
5	5	−	8	2/8	25
5	10	−	7	3/7	43
5	15	−	6	3/6	50
5	15	+	52	14/33	42
10	10	+	38	14/33	42
15	15	+	95	7/12	58

H3 cells (*nit1-305 pf-14*) were grown in SGII-NH_4 medium to ≈5 × 10^6 cells per ml and harvested by centrifugation at 5000 × *g* for 5 min. Half the cells were resuspended in SGII-NO_3 liquid medium and transformed without added PEG as described; the other half was incubated in 5 ml of autolysin for 1 hr. After autolysin treatment, cells were resuspended in SGII-NO_3 liquid medium and transformed. Motility was measured by growing cells in liquid 1/2R-NO_3 medium (8) and examining cultures for swimming cells with an Olympus inverted microscope.

DISCUSSION

The nuclear transformation procedure described above for *Chlamydomonas* differs from the procedure developed for yeast (12) in several ways. The yeast procedure uses intact, cell-wall-containing yeast cells, whereas the procedure for *Chlamydomonas* is not efficient unless the cell wall is removed, either by mutation or by treating cells with autolysin. Extending the agitation period to several minutes for cell-wall-containing strains did not increase the number of transformants recovered. Although glass beads are not required for transformation of *Chlamydomonas*, yeast transformants were not recovered when glass beads were omitted. PEG greatly enhanced transformation efficiency with cell-wall-deficient *Chlamydomonas* cells, but in preliminary experiments did not enhance transformation in yeast (M. C. Costanzo and T. D. Fox, personal communication). Finally, the yeast procedure does not appear to be effective for mitochondrial transformation (M. C. Costanzo and T. D. Fox, personal communication); we have used a modification of the procedure described above to transform the chloroplast genome of *Chlamydomonas* (K. L. Richards and K.L.K., unpublished observations).

This method for introducing DNA into the nuclear genome of *Chlamydomonas* has several advantages over the particle gun. It is simple, inexpensive, and does not require access to specialized equipment. The transformants so far analyzed appear to integrate the introduced DNA at lower copy number than those generated by the particle gun, an advantage for some applications. Moreover, the method is highly reproducible both within and between experiments. Most importantly, the efficiency of transformation using this method greatly exceeds that of the particle gun. With the present efficiency, on the order of 10^3 transformants per μg of DNA, experiments that require high rates of transformation should now be possible. These include gene disruption and gene replacement experiments, which require homologous recombination. In higher eukaryotes, the ratio of homologous to nonhomologous recombination events ranges from 10^{-2} to 10^{-5} (23–27).

With the present transformation rates, it may be possible to clone genes by complementing stable mutations. Because of the large number of interesting mutants available in *Chlamydomonas* (19), this capability would allow the isolation and characterization of many genes with known phenotypic effects. Because the rate of cotransformation is so high, any cloned gene can readily be introduced into *Chlamydomonas*. The combination of an efficient nuclear transformation system with the ability to genetically manipulate the chloroplast genome by transformation (refs. 6 and 7; K. L. Richards and K.L.K., unpublished work) should make *Chlamydomonas* an ideal organism for studies of chloroplast–nuclear interactions.

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1. Luck, D. J. L. (1984) *J. Cell Biol.* **98,** 789–794.
2. Lefebvre, P. A. & Rosenbaum, J. L. (1986) *Annu. Rev. Cell Biol.* **2,** 517–546.
3. Snell, W. J. (1985) *Annu. Rev. Plant Physiol.* **36,** 287–315.
4. Levine, R. P. & Goodenough, U. W. (1970) *Annu. Rev. Genet.* **4,** 397–408.
5. Rochaix, J.-D. & Erickson, J. (1988) *Trends Biochem. Sci.* **13,** 56–59.
6. Boynton, J. E., Gillham, N. W., Harris, E. H., Hosler, J. P., Johnson, A. M., Jones, A. R., Randolph-Anderson, B. L., Robertson, D., Klein, T. M., Shark, K. B. & Sanford, J. C. (1988) *Science* **240,** 1534–1537.
7. Blowers, A. D., Bogorad, L. B., Shark, K. & Sanford, J. C. (1989) *The Plant Cell* **1,** 123–132.
8. Kindle, K. L., Schnell, R. A., Fernández, E. & Lefebvre, P. (1989) *J. Cell Biol.* **109,** 2589–2601.
9. Day, A., Debuchy, R., van Dillewijn, J., Purton, S. & Rochaix, J.-D. (1990) *Physiol. Plant.*, in press.
10. Debuchy, R., Purton, S. & Rochaix, J.-D. (1989) *EMBO J.* **8,** 2803–2809.
11. Fernández, E., Schnell, R., Ranum, L. P. W., Hussey, S. C., Silflow, C. D. & Lefebvre, P. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6649–6453.
12. Costanzo, M. C. & Fox, T. D. (1988) *Genetics* **120,** 667–670.
13. Williams, B., Velleca, M. A., Curry, A. M. & Rosenbaum, J. (1989) *J. Cell Biol.* **109,** 235–245.
14. Fernández, E. & Matagne, R. F. (1984) *Curr. Genet.* **8,** 635–640.
15. Sosa, F. M., Ortega, J. & Barea, J. L. (1978) *Plant Sci. Lett.* **11,** 51–58.
16. Davies, D. R. & Plaskitt, A. (1971) *Genet. Res.* **17,** 33–43.
17. Huang, B., Piperno, G., Ramanis, Z. & Luck, D. J. L. (1981) *J. Cell Biol.* **88,** 80–88.
18. Sager, R. & Granick, S. (1953) *Ann. N.Y. Acad. Sci.* **56,** 831–838.
19. Harris, E. H. (1989) in *Chlamydomonas Handbook* (Academic, New York), pp. 47, 593–594.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 90–91.
21. Widom, J. & Baldwin, R. L. (1980) *J. Mol. Biol.* **144,** 431–453.
22. Snell, W. J., Eskue, W. A. & Buchanan, M. J. (1989) *J. Cell Biol.* **109,** 1689–1694.
23. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. (1988) *Nature (London)* **336,** 348–352.
24. Paszkowski, J., Baur, M., Bogucki, A. & Potrykus, I. (1988) *EMBO J.* **7,** 4021–4026.
25. Lin, F.-L., Sperle, K. & Sternberg, N. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1391–1395.
26. Doetschman, T., Maeda, N. & Smithies, O. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8583–8587.
27. Zimmer, A. & Gruss, P. (1989) *Nature (London)* **338,** 150–155.