ligand to its receptor, activation of tyrosine kinase, and the stimulation of DNA synthesis by both the AA and BB forms of PDGF. The native mouse receptor and the human receptor encoded by the cDNA sequence reported here are functionally identical in mediating these activities. These findings show that the AA form of PDGF is an authentic growth factor that acts through the same mitogenic pathways as previously studied BB and AB forms.

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18. An expression vector containing the human cDNA sequence under transcriptional control of the SV40 early promoter and the expression vector pSV2neo carrying the gene that confers resistance to neomycin were used to cotransfect CHO cells by the calcium precipitate technique. After transfection, the cells were cultured in the presence of 400 μg/ml of G-418 (a neomycin analog). G-418–resistant colonies were picked after 2 weeks in selection media. Each colony was assayed as described (*30*) for the expression of PDGF receptor by Western blot with an antibody to the receptor that specifically recognizes sequences in the carboxyl-terminal region of the receptor (29). A clone expressing the highest amount of receptor (CHO-HR5) was used in these experiments. The 195-kD protein recognized by this antibody has a size of 195 kD.
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Chloroplast Transformation in *Chlamydomonas* with High Velocity Microprojectiles

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Bombardment of three mutants of the chloroplast *atpB* gene of *Chlamydomonas reinhardtii* with high-velocity tungsten microprojectiles that were coated with cloned chloroplast DNA carrying the wild-type gene permanently restored the photosynthetic capacity of the algae. In most transformants of one of the mutants, a fragment with a 2.5-kilobase deletion was restored to normal size by a homologous replacement event; in about 25 percent of the transformants the restored restriction fragment was 50 to 100 base pairs smaller or larger than that of wild type. About one-fourth of the transformants of this mutant contained unintegrated donor plasmid when first examined. This plasmid persisted in four different transformants after 65 cell generations of continuous liquid culture but was lost from all transformants maintained on plates of selective medium. The restored wild-type *atpB* gene remains in all transformants as an integral part of the chloroplast genome and is expressed and inherited normally.

IN VIEW OF THE EASE WITH WHICH nuclear genes of plant and animal cells can be transformed, the lack of reliable methods for introducing genes into mitochondria or chloroplasts is noteworthy. While mitochondrial transplantation by microinjection has been successful (*1*), previous accounts of DNA mediated transformation of yeast mitochondria (*2*) and tobacco chloroplasts (*3*) have not been verified in the literature. However, indirect "transformation" of chloroplasts or mitochondria has been accomplished by introducing constructs into the nucleus carrying an organelle gene with an added amino-terminal transit sequence to target the protein to the organelle (*4*). Direct transformation of chloroplasts and mitochondria has been difficult because both organelles are bounded by double membrane envelopes that may hinder nucleic acid transport (*5*) and because many genetically polyploid organelles are present in most eukaryotic cells. We report here that the newly developed "biolistic" DNA delivery process utilizing high velocity microprojectiles (*6*) circumvents these problems, probably by direct introduction of many copies of a gene into the chloroplast. This process is also proving effective for introducing DNA into the nuclei of higher plants (*7*) and for nuclear (*8*) and mitochondrial (*9*) gene transformation of yeast.

The single cup-shaped chloroplast of the unicellular green alga *Chlamydomonas reinhardtii* is a favorable target for bombardment by DNA-coated microprojectiles, since it lies adjacent to the plasma membrane along most of the periphery of this 10-μm diameter cell (*5*). For transformation recipients, we used three photosynthetically defective mutants (Fig. 1A) with lesions in the *ac-u-c* locus (*10–12*), which defines the chloroplast *atpB* gene encoding the β subunit of the CF_1 complex of the chloroplast adenosine triphosphate (ATP) synthase. The mutant *ac-u-c-2-21* (CC-373) has a 2.5-kb deletion in the Bam 10 restriction fragment spanning this gene; the deletion extends from the 3′ half of *atpB* into the 22-kb inverted repeat of the chloroplast genome.

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In *ac-u-c-2-43* (CC-1015), a 13-kb deletion starts in the 3′ half of *atpB* and extends through one set of ribosomal RNA (rRNA) genes. The mutant *ac-u-c-2-29* (CC-440) has an AT → CG transversion at position 462 of the *atpB* gene. Neither deletion mutation reverts to photosynthetic competence or accumulates any *atpB* transcript. The point mutant makes a full-sized nonfunctional polypeptide and reverts to wild type at a frequency of 1×10^{-8}. Each mutant was transformed by bombarding monolayers of cells on petri plates of permissive (acetate-containing) medium (*13*) with tungsten microprojectiles coated with wild-type Bam 10 cloned in pBR313 (plasmid P-17). We estimate that each 1.2-μm diameter microprojectile carried 20 to 50 copies of the 16.3-kb P-17 plasmid and that 10^7 to 10^8 microprojectiles bombarded 5.7×10^6 cells on each plate. After incubation for 18 to 24 hours in the dark, cells were replated on selective (minimal) medium in bright light (*13*). Green colonies were first seen on a lawn of dead, bleached cells after 7 to 10 days. Presumptive transformants maintained on minimal medium in bright light grew at normal rates, attesting to their restored photosynthetic capacity.

The highest frequencies of *ac-u-c-2-21* transformants (1.9×10^{-6} to 4.2×10^{-6}, or about 10 to 20 transformants per microgram of DNA applied) were obtained (Table 1) by bombarding cells on permissive medium with P-17 DNA–coated 1.2-μm diameter microprojectiles [gunpowder charge No. 1 was used (*6*)]. Smaller particles (0.6 μm) yielded no transformants in 1.14×10^7 cells and charge Nos. 2 and 5 gave transformation frequencies of 0.44×10^{-6} and 0.04×10^{-6}, respectively. No spontaneous revertants were detected in 1.3×10^9 untreated mutant cells plated on selective medium. Bombardment of 1.14×10^7 recipient cells with uncoated particles or with particles coated with pBR313 DNA yielded no transformants. Particles coated with Bam 10 isolated from P-17 and purified by electrophoresis on two sequential agarose gels were also ineffective (no transformants in 2.3×10^7 cells bombarded). Transformation frequencies were reduced 10- to 100-fold when recipient cells were bombarded directly on selective rather than permissive medium (Table 1).

Analysis of total cell DNA from 54 representative transformants of *ac-u-c-2-21* revealed that all had lost the 5.1-kb Bam fusion fragment of the mutant (ΔBam 10), and 39 had acquired a normal 7.6-kb Bam 10 fragment (Fig. 1B). The P-17 probe hybridizes to both Bam 10, which contains *atpB*, and the partially homologous fragment, Bam 9, from the other copy of the inverted repeat. Twelve transformants (for example, T12A-5 and T15B-1) had Bam 9 and Bam 10 fragments 50 to 100 bp smaller

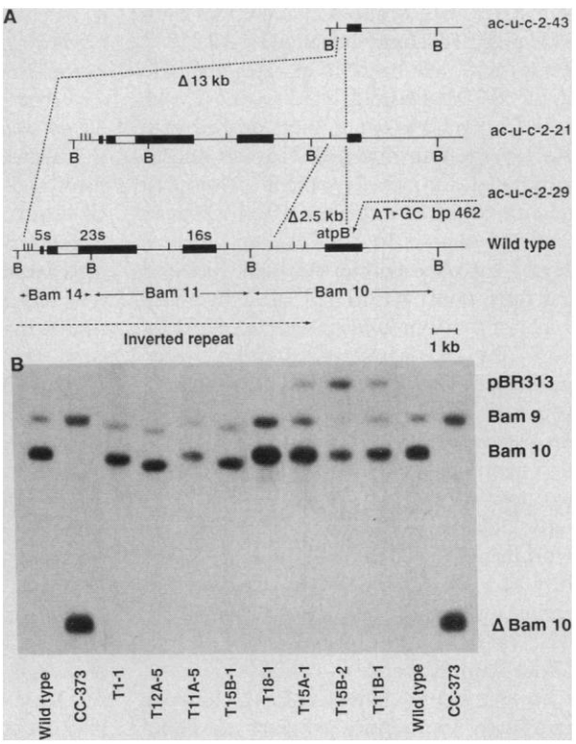

Fig. 1. (**A**) Restriction maps of the chloroplast *atpB* deletion mutants *ac-u-c-2-21* and *ac-u-c-2-43* and the *atpB* point mutant *ac-u-c-2-29* used as recipients for transformation. B, Bam HI restriction sites; vertical bars above each line represent short dispersed repeat sequences. Dotted lines define the deleted sequences in the two mutations compared to wild type, and an arrow shows the endpoint of the 22-kb inverted repeat. (**B**) Autoradiogram of Southern blot of total cellular DNA from eight *ac-u-c-2-21* transformants restricted with Bam HI, separated on a 0.8% agarose gel and probed with ^{32}P nick-translated DNA of P-17, the donor pBR313 plasmid containing the chloroplast fragment Bam 10. Total cellular DNA was prepared (*22*) from representative isolates grown photosynthetically (*13*) in 100-ml HS shake cultures bubbled with 5% CO_2 in bright light (200 μE/m² per second).

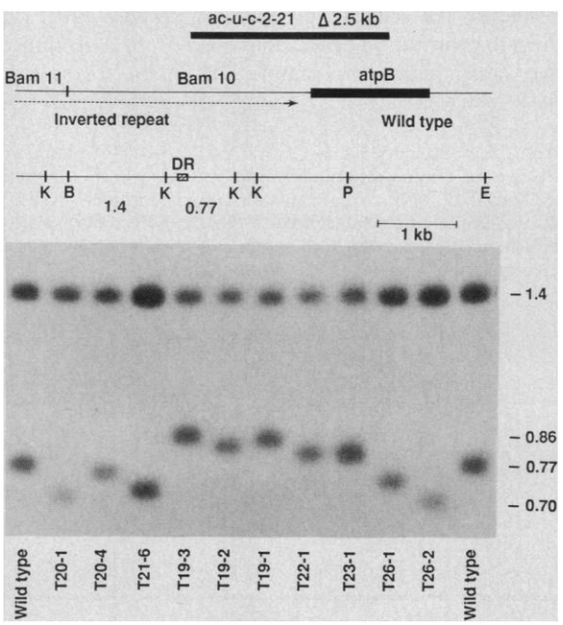

Fig. 2. Autoradiogram of Southern blot of total DNA from selected transformants restricted with Kpn I, separated on a 1% agarose gel and probed with nick-translated P-17 DNA. Restriction sites: K, Kpn I; B, Bam HI; P, Pst I; and E, Eco RI. A 12-bp sequence reiterated 16 times as a tandem repeat (*14*) is indicated by a striped box (DR).

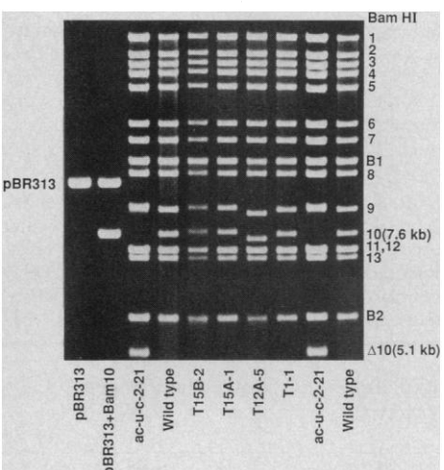

Fig. 3. Chloroplast DNA from *ac-u-c-2-21* transformants grown in liquid minimal medium after several prior transfers on solid medium, purified on NaI density gradients (*23*), restricted with Bam HI, separated on 0.8% agarose gel, and stained with ethidium bromide. Fragments smaller than 5 kb are not shown, but are in normal stoichiometry on the original gel.

and three had fragments 50 to 100 bp larger than those of the wild type. These results suggest that deletions or duplications occurred in the inverted repeat region of Bam 10 during insertion and were subsequently copy-corrected (*12*) to Bam 9. The size differences were confined to a 0.77-kb Kpn I fragment, within which the 2.5-kb deletion of *ac-u-c-2-21* terminates (Fig. 2). This fragment contains a 12-bp repeat sequence reiterated 16 times in tandem (DR) in the strain (CC-277 *cw-15 mt*$^+$) from which the P-17 insert was derived (*14*). The small deletions and duplications likely result from unequal

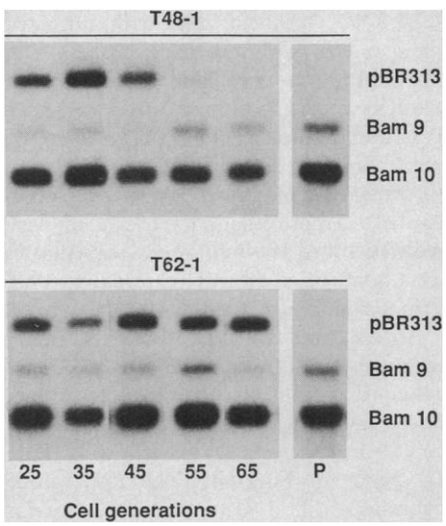

Fig. 4. Autoradiogram of a Southern blot of cellular DNA from two transformants sampled after various times in liquid medium, digested with Bam HI, separated on a 0.8% agarose gel, and probed with nick-translated P-17. Total cell DNA was prepared from four original cultures grown to stationary phase (1×10^7 to 2×10^7 cells per milliliter) and from four sequential subcultures of each transformant started at a density of 1×10^4 and harvested at stationary phase. Lane P represents cellular DNA from cells grown in liquid minimal medium for 25 generations and subcultured twice on solid minimal medium.

pairing and crossing-over between the repeat sequences of the transforming DNA and those of the fusion fragment of the recipient. Bam HI digests of purified chloroplast DNA from four transformants showed normal restriction patterns with stoichiometric amounts of Bam 10 (Fig. 3).

Thirteen of 54 *ac-u-c-2-21* transformants examined initially retained varying amounts of the transforming plasmid P-17, but all lost the plasmid during subculture on selective plates. Three lines of evidence indicate that P-17 was replicating autonomously in cells of these 13 isolates. (i) In Bam HI digests of total cell DNA probed with P-17 (Fig. 1B, T15A-1, T15B-2, and T11B-1) or with pBR313 alone, linearized pBR313 is seen as an 8.7-kb band. In all cases, the ratio of the 8.7-kb plasmid band to the 7.6-kb Bam 10 band was <1 in blots probed with P-17, suggesting that pBR313 was not incorporated into the recipient chloroplast genome together with Bam 10. (ii) Digests of these same DNAs with Pst I, which should cut once within the Bam 10 insert and three times within pBR313, gave fragments of the approximate sizes expected for P-17 (8.9, 5.0, 1.8, and 0.6 kb) when probed with pBR313. The higher molecular weight fragments predicted to result from integration of the plasmid into the chloroplast genome on either side of Bam 10 were not observed. (iii) In Sal I digests of the same DNA probed with pBR313, a single hybridizing band representing the linearized form of P-17 was seen, whereas no hybridization was detected in wild type, in *ac-u-c-2-21*, or in transformants that lacked the 8.7-kb Bam HI band.

To examine the kinetics of P-17 loss, we transferred four entire colonies to liquid minimal medium as soon as they became visible on the selective plates and allowed them to grow for 65 generations (Fig. 4). In two transformants (for example, T48-1), the ratio of pBR313 to Bam 10 declined precipitously, and only trace amounts of plasmid were present after 65 generations. In a third transformant (T62-1), a constant ratio persisted throughout, and in the fourth the ratio dropped gradually. All four transformants lost P-17 when transferred twice on solid medium after an initial 25 generations in liquid culture. Integration of wild-type Bam 10 likely occurred well before the first culture of each transformant was harvested, since only trace amounts of the characteristic fusion fragment of the recipient were detectable by then. Hence in liquid culture P-17 must replicate in the transformed cells since it persists for many generations. Because neither mapped chloroplast origin of replication (*15*) is located in Bam 10, a pBR313 sequence may function as an origin in *Chlamydomonas*.

Absence of the 5.1-kb mutant fusion fragment and integrated pBR313 sequences in all *ac-u-c-2-21* transformants suggests that direct replacement of the missing chloroplast sequences by the corresponding wild-type sequences occurs. The most likely possibility is a double crossover or gene conversion event between regions of homology beyond the ends of the mutant fusion fragment and the wild-type Bam 10 fragment carried by P-17. A high frequency of direct homologous replacement events with no integration of plasmid sequences is also seen in unicellular cyanobacteria transformed with homologous donor sequences (*16*).

Four transformants of *ac-u-c-2-21* had normal levels of both the α and β subunits of the CF_1 complex on their thylakoids (Fig. 5). In contrast, thylakoids of *ac-u-c-2-21* lacked detectable α and β subunits as do other mutants affecting the ATP synthase (*10*). Thus the introduced *atpB* gene appears to function normally and to restore the entire ATP synthase complex. The intro-

Table 1. Frequency of transformation of *atpB* deletion and point mutants obtained using P-17, a pBR313 plasmid containing the 7.6-kb wild-type Bam 10 chloroplast DNA fragment. HS, minimal medium selective for photosynthetically competent cells; HSHA, nonselective medium containing acetate as a carbon source (*10*). Cell numbers in samples bombarded with P-17 DNA are corrected for estimated number of cells bombarded (5.7×10^6). Frequencies in experiments in which cells were bombarded on HSHA medium and then transferred to HS are corrected for plating efficiency (about 25%) and estimated number of cell divisions between bombardment and replating. Bombardment in all cases was with 1.2-μm tungsten particles and charge No. 1. The range of frequencies observed for *ac-u-c-2-21* HSHA → HS represents four separate experiments.

Recipient genotype	Transforming DNA	Plating medium	Total cells ($\times 10^7$)	Photosynthetic colonies	
				Number	Frequency ($\times 10^{-6}$)
ac-u-c-2-21	P-17	HS	6.8	6	0.09
	P-17	HSHA → HS	23.6	299	1.9 to 4.2
	None	HS	130.0	0	<0.0008
ac-u-c-2-43	P-17	HSHA → HS	5.7	434	7.6
	None	HS	28.0	0	<0.003
ac-u-c-2-29	P-17	HSHA → HS	5.7	27	0.5
	None	HS	28.0	4*	0.014

*These four colonies are presumptive revertants of the *ac-u-c-2-29* point mutation.

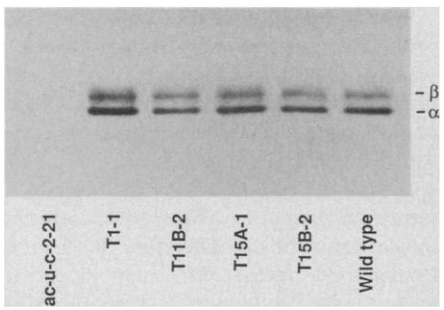

Fig. 5. Isolated thylakoid proteins from three *ac-u-c-2-21* transformants probed with a mixture of antibodies to the α and β subunits of ATP synthase. Thylakoids were isolated (*24*) from cells grown in tris-acetate-phosphate (TAP) medium (*25*) in dim light, and proteins were separated on SDS-urea polyacrylamide gels (*11*). After the gels were washed (three times in 192 m*M* glycine, 25 m*M* tris, and 20% methanol), they were electroblotted to nitrocellulose in the same buffer and probed with a 1:1000 dilution of polyclonal antibodies from the α CF_1 subunit of *C. reinhardtii* and the β CF_1 subunit from spinach and ^{125}I-labeled protein A (*26*). The *C. reinhardtii* α (57 kD) and β (53 kD) subunits are reversed in position in SDS-urea gels (*27*).

duced Bam 10 fragment was inherited in the expected non-Mendelian (>95% uniparental) fashion (*5*) typical of chloroplast genes in progeny of three representative transformants mated to a strain with physically distinguishable Bam 9 and Bam 10 fragments (*17*). The restored Bam 10 fragment from the *mt*$^+$ transformants was transmitted uniparentally to 24 of 26 meiotic progeny examined (four complete tetrads and ten randomly selected products from different tetrads). One tetrad showed non-Mendelian biparental transmission, with two parental and two recombinant progeny. Genetic analysis of 629 progeny from crosses of these same three transformants to an *mt*$^-$ isolate of *ac-u-c-2-21* also demonstrated predominantly uniparental inheritance of the photosynthetically competent phenotype of the transformants.

We have also transformed two other *atpB* mutants to photosynthetic competence with P-17 (Table 1). A threefold higher frequency of transformants was seen for mutant *ac-u-c-2-43*, which has a 13-kb deletion, and a fivefold lower frequency for *ac-u-c-2-29*, which contains a point mutation, when compared to the 2.5-kb deletion mutation *ac-u-c-2-21* in the same experiment. The defective β subunit synthesized by *ac-u-c-2-29* may compete with its normal counterpart for assembly into CF_1. This may reduce the expression of photosynthetic competence in this recipient and make selection and recovery of transformants more difficult. Three of the four *ac-u-c-2-29* transformants examined contained plasmid sequences, and the normal-sized Bam 10 fragment of this point mutant was unchanged in all four. All 26 transformants of *ac-u-c-2-43* examined contained fragments of the same size as wild-type Bam 10 and lacked the ~4-kb mutant fragment composed of the fused ends of Bam 10 and Bam 14 (Fig. 1A). Only one of the 26 transformants contained plasmid sequences. Since the chloroplast genome of *ac-u-c-2-43* lacks the end of Bam 10 terminating in the inverted repeat (Fig. 1A), it has no region of homology with which one end of the wild-type donor Bam 10 fragment can pair. Instead, pairing might occur between short repeat elements (*12*) found in the portion of Bam 14 remaining in *ac-u-c-2-43* and homologous short repeat sequences in the wild-type Bam 10 fragment carried by P-17 (Fig. 1A, short vertical bars). This pairing could allow integration of the entire *atpB* gene and a short segment of the inverted repeat. Subsequent copy correction (*12*) could restore both inverted repeat copies to normal size.

We have also obtained transformants (*18*) for a missense mutation in the chloroplast *rbcL* gene, which encodes the large subunit of ribulose bisphosphate carboxylase (*19*), and for a symmetrical deletion mutant in the *psbA* gene, which encodes the 32-kD Q_B herbicide-binding protein (*20*). Thus, transformation is possible for chloroplast genes of *C. reinhardtii* located in either the single-copy or inverted repeat regions of the organelle genome. The biolistic process may prove useful for transforming chloroplasts of higher plants even though each cell contains many chloroplasts considerably smaller than the single chloroplast of *Chlamydomonas*. The successful transformation of yeast mitochondria (*9*) suggests that having several small, genetically polyploid organelles may not limit transformation, provided that multiple copies of the donor DNA can be delivered and sufficiently strong selection for the donor gene can be applied. Thus far, successful transformation of chloroplasts has depended upon the introduction of cloned wild-type genes into organelle mutants with stringent defects in photosynthesis. We are exploring the use of cloned genes from antibiotic-resistant (*5*) and herbicide-resistant mutants (*20*) to transform wild-type sensitive cells to resistance. While few, if any, deletions or point mutations are known that eliminate organelle gene functions in higher plants or animals, mutants resistant to antibacterial antibiotics (*1*) and herbicides (*21*) may provide an alternative means of selecting transformants. If not, use of a foreign dominant selectable marker may provide sufficiently stringent selection for isolation of chloroplast or mitochondrial transformants.

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13. Mutant cells were grown at Duke on solid or liquid HSHA (acetate) medium (*10*) under dim light (3 μE/m^2 per second photosynthetically active radiation), harvested at mid-log phase (2×10^6 to 4×10^6 per milliliter) and resuspended in HSHA at 1.14×10^8 per milliliter. Resuspended cells (0.5 ml) were added to 0.5 ml of 0.2% soft agar (Difco) at 42°C and mixed gently; 0.25-ml portions were then quickly dispensed onto 5-cm petri plates containing 4-day-old HS or HSHA medium. Plates were swirled rapidly to create a uniform monolayer of cells, allowed to dry in darkness for 2 hours, shipped overnight to Cornell for bombardment, and returned to Duke the next day. Cells bombarded on HSHA were resuspended in 0.7 ml of liquid HS (minimal) medium and replated on 15-cm petri plates of HS. All plates were incubated in bright light (80 μE/m^2 per second) to select photosynthetically competent colonies.
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Mitochondrial Transformation in Yeast by Bombardment with Microprojectiles

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The genetic transformation of mitochrondria and chloroplasts has been an intractable problem. The newly developed "biolistic" (biological ballistic) process was used to deliver DNA into yeast cells to stably transform their mitochondria. A nonreverting strain, which is respiratory deficient because of a deletion in the mitochondrial *oxi3* gene, was bombarded with tungsten microprojectiles coated with DNA bearing sequences that could correct the *oxi3* deletion. Respiratory-competent transformants were obtained in which the introduced *oxi3* DNA is integrated at the homologous site in the mitochondrial genome. Organelle genomes can now be manipulated by molecular genetic techniques in the same way as nuclear genomes.

THE ABILITY TO TRANSFORM CELLS with foreign DNA has proved to be an invaluable tool of molecular genetic analysis. However, many important questions in mitochondrial molecular biology remain unapproachable because of the lack of a transformation system. This inability has, for example, stymied systematic mutational studies of cis-acting sequences in the mitochondrial genome and of mitochondria-encoded proteins. Previous attempts at mitochondrial transformation, both published (*1*) and unpublished, have been unsuccessful, nonreproducible, or have lacked unambiguous molecular proof. One presumed difficulty has been in introducing DNA across the outer and inner mitochondrial membranes. The invention of the biolistic process by Sanford and co-workers (*2*) suggested a new approach to mitochondrial transformation. The procedure entails coating small (1-μm) tungsten projectiles with the nucleic acid of interest. These microprojectiles are accelerated in a special device to velocities sufficient to penetrate cells (*2*), as has recently been demonstrated by the introduction of biologically active DNA and RNA into various plant cells (*3*). We reasoned that this process might deliver DNA directly into the mitochondria by physically disrupting the mitochondrial membranes. We chose to test the applicability of this procedure to mitochondrial transformation by using the yeast *Saccharomyces cerevisiae*. Yeast are well suited for this effort since they do not require functional mitochondria for growth. In addition, the yeast mitochondrial genome is highly recombinogenic and has been extensively characterized at the genetic and molecular levels. Moreover, a wide variety of mitochondrial mutations are available [reviewed in (*4*)]. We developed a method for high-efficiency transformation of yeast nuclear genes by the biolistic process (*5*); our method proved to be a prerequisite for successful mitochondrial transformation.

Our approach to mitochondrial transformation was to attempt to correct a molecularly defined deletion in a mitochondrial gene with an identifiable foreign mitochondrial DNA (mtDNA) sequence. By using a mitochondrial lesion which makes the strain unable to respire (that is, unable to grow on a nonfermentable carbon source such as glycerol), we could select for restoration of respiratory competence. This approach allowed us to preclude any apparent transformants that might arise from contamination or any unforeseen suppression events. Use of a deletion strain also obviated any concern about interference from a defective protein. Co-transformation with a nuclear plasmid was used to increase the probability of recovering stable transformants. We reasoned that initially only one or a few of the approximately 50 mitochondrial chromosomes per yeast cell are likely to be corrected in the transformant. Therefore, by first selecting for a nuclear marker, we hoped to allow for the production of segregants with high copy numbers of transformed mitochondria prior to selection for restoration of mitochondrial function. Co-transformation also provided a measure of mitochondrial versus nuclear transformation frequencies.

We used a respiratory-deficient (mit^-) strain, 947/PA5, as the recipient in mitochondrial transformation. This strain harbors a 2547-bp deletion within the "long" form of the mitochondrial *oxi3* gene, which encodes the largest subunit of cytochrome oxidase (COXI) (Fig. 1) (*6, 7*). Briefly, the mitochondrial mutation in this strain was originally isolated as a spontneous suppressor of the respiratory-deficient *oxi3* splicing–defective mutant C1036. The strain C1036 does not express COXI because it contains a termination codon within the open reading frame (ORF) of the *oxi3* intron 1 (aI1) (Fig. 1). Like a number of other introns within the yeast mitochondrial genome, the aI1 ORF is in-frame with its upstream exon, and the fusion gene product is a trans-acting splicing factor (maturase) required for splicing of that intron (*8*). The mitochondrial lesion designated PA5 arose as a result of a crossover that joined the coding sequence from position 967 of aI1 to position 3514 of aI2 (*9*) (Fig. 1). This recombination

Table 1. Cytochrome oxidase activities (nanomoles of O_2 per minute per milligram of protein) in wild-type and transformed strains. The wild-type strain, (COP161, ρ^+), the mit^- recipient (947/PA5), and the six transformed strains were grown in medium containing 2% raffinose as the carbon source to a cell density of 1×10^7 to 3×10^7 cells per milliliter. The cells were pelleted and washed with distilled water and protein extracted as previously described (*21*). Cytochrome oxidase assays (*22*) and protein determination (*23*) were performed on the supernatant. Activities are expressed as means ± 2 SD and are based on three repetitions for each determination.

Strain	Cytochrome oxidase activity (nmol/min-mg)
COP161	331 ± 92
947/PA5	0
947/PA5-1	210 ± 6.5
947/PA5-3	210 ± 13
947/PA5-8	257 ± 17
947/PA5-9	269 ± 29
947/PA5-10	217 ± 24
947/PA5-13	204 ± 9.0

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