Localization of the *nic-7*, *ac-29* and *thi-10* Genes Within the Mating-Type Locus of *Chlamydomonas reinhardtii*

Patrick J. Ferris

Department of Biology, Washington University, St. Louis, Missouri 63130

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ABSTRACT

The tight linkage observed between the mating-type (*mt*) locus of *Chlamydomonas reinhardtii* and three auxotrophic mutations—*nic-7* (nicotinamide-requiring), *ac-29* (acetate-requiring), and *thi-10* (thiamine-requiring)—has led to the hypothesis that recombination is suppressed in the *mt* region. The physical location of these three genes has been established by transformation with sets of cloned DNA from the *mt* region. They lie to the left and right of the highly rearranged (R) domain of the *mt* locus, which has been proposed to be responsible for the recombinational suppression in the region. The cloned *nic-7*$^+$ *and thi-10*$^+$ genes will be useful as selectable markers for cotransformation experiments.

THE mating-type (*mt*) locus of *Chlamydomonas reinhardtii* is a region of linkage-group VI that controls the expression of gametic traits, zygote development, and the uniparental inheritance of chloroplast and mitochondrial DNA (GOODENOUGH *et al.* 1995). Thirteen genetic loci have been identified that are tightly linked to *mt*, many of which dictate traits that are unrelated to life-cycle transitions (FERRIS and GOODENOUGH 1994). GILLHAM (1969) first proposed that such linkages might be the fortuitous consequence of a recombinational suppression that serves to maintain the linkage of mating-type-related genes and "spreads" to include flanking sectors of the chromosome.

A 1.1-Mb chromosome walk spanning the *mt* locus has recently been completed (FERRIS and GOODENOUGH 1994). The two *mt* loci (*mt*$^+$ and *mt*$^-$) were found to contain a domain (the R domain) of 190 kb in which the DNA is highly rearranged and inverted, and it was postulated that this configuration suppresses recombination not only in the R domain but also in the surrounding chromosome. This postulate predicts that *mt*-linked genes unrelated to the life-cycle will localize within the (525 kb) centromere-proximal (C domain) and the (110 kb) telomere-proximal (T domain) DNA flanking the R domain (Figure 1).

The present study reports the use of complementation to localize three such genes. The *nic-7* (nicotinamide-requiring) mutation, previously reported to lie <0.3 cM centromere-distal of *mt* (SMYTH *et al.* 1975), has been localized in the T domain, 80 kb from the left border of the R domain (Figure 1), while the *ac-29* (acetate-requiring) mutation, also in the T domain, is located 40 kb from the left R border. The *thi-10* (thiamine-requiring) mutation has been localized in the C domain, 145 kb from the right border of the R domain. These results confirm the GILLHAM hypothesis by demonstrating that these genes are separated by larger physical distances than would be expected from genetic data. The genomic clones for *nic-7* and *thi-10* can be used as selectable markers in *C. reinhardtii* cotransformation experiments.

Corresponding author: Patrick J. Ferris, Department of Biology, Campus Box 1229, Washington University, St. Louis, MO 63130.
E-mail: ferris@wustlb.wustl.edu

MATERIALS AND METHODS

Strains and growth conditions: The strains used are listed in Table 1. Genetic evidence has suggested that *nic-14* and *nic-17* are alleles of *nic-7*; transformation data supporting this assumption are described in this paper. Strains were maintained on solid TAP medium, supplemented with 4 μg/ml nicotinamide or 5 μg/ml thiamine as needed. Sueoka's high salt medium (HSM) was used whenever an acetate-deficient medium was required (HARRIS 1989). Crosses were carried out using standard protocols (HARRIS 1989).

Transformations: The glass bead method of KINDLE (1990) was used for Chlamydomonas transformations. To transform *nic-7* alleles, mutant cells were grown in 50 ml TAP containing 4 μg/ml nicotinamide until they reached a density of 2–4 × 10^6/ml. Approximately 2 × 10^7 cells were harvested and incubated with 4 ml (see below) gametic lytic enzyme (GLE) (KINOSHITA *et al.* 1992) for 60 min to remove the cell walls. The effectiveness of the GLE treatment was monitored by assaying the sensitivity of the cells to lysis in 0.1% NP-40; incubation in a fresh aliquot of GLE was performed as needed. Protoplasts were harvested in a 15-ml polypropylene tube (Corning) and resuspended in 300 μl TAP, 100 μl 20% PEG-8000. Glass beads (0.5 mm, 300 mg) and the transforming DNA (0.5–2 μg) were added and the cells vortexed for 15 sec at the highest setting using a Vortex-Genie mixer. An additional 600 μl of TAP were added and the cells divided among three plates. Cells were either plated directly onto TAP medium containing 15 μl/liter 3-acetylpyridine (Sigma), which is toxic to nicotinamide-requiring cells (HARRIS 1989), or else were plated onto TAP and 0.5–1 μl 3-acetylpyridine was spotted onto the plate the next day. Both protocols gave equivalent yields of Nic$^+$ transformants, which become visible after 4–5 days. It is also possible to distinguish transformed colonies by plating onto TAP without 3-acetylpyridine, but

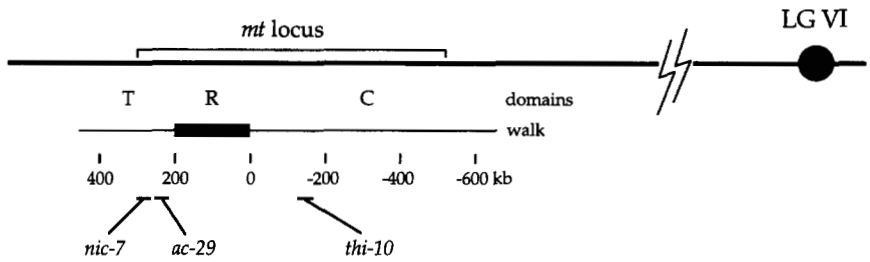

FIGURE 1.—Map of the *C. reinhardtii mt* locus. The left arm of linkage group VI is depicted, showing the location of the chromosome walk covering the *mt* locus. The positions of the T, R, and C domains are shown, as well as the approximate locations of the three *mt*-linked markers, *nic-7, ac-29* and *thi-10.*

this approach yields a pale green background of untransformed cells. In control experiments, revertants were observed, at low frequency, with all three *nic-7* alleles.

Essentially the same protocol was used to transform *thi-10* cells. Because untransformed *thi-10* cells persist as a pale green lawn, fewer cells (1×10^7) were subjected to transformation and these were spread onto five TAP plates to reduce the background.

The *ac-29* strains are not, as their name suggests, acetate requiring, so transformants cannot be selected by screening for phototrophic growth. However, the mutants are yellow in color and grow more slowly than wild type, whether or not the medium contains acetate. There are two extant alleles, *ac-29* and *ac-29a,* that fail to complement in diploids (data not shown). The yellow color of *ac-29a* is easier to distinguish from the color of wild type than is the color of *ac-29,* so the former was used for most experiments. Reconstruction experiments in which wild-type cells were mixed with *ac-29a* cells at a ratio of approximately 1:10,000 indicated that green colonies could not be reliably identified against the lawn of yellow cells when plated at 10^6 cells per plate (plating at lower densities would require the use of large numbers of plates). However, if the mixture of cells was grown in liquid TAP, and an inoculum regularly transferred to fresh medium, the faster growing wild-type cells eventually predominated. Therefore, transformation of *ac-29a* was performed by vortexing with glass beads as for *nic-7,* allowing the cells to grow in liquid TAP medium to saturation, and then transferring an aliquot to fresh medium. The transfer was repeated 8–10 times, with aliquots of $\sim 10^5$ cells plated at intervals to determine the presence of any green cells.

GLE was prepared as follows. CC-620 (mt^+) and CC-621 (mt^-) gametes were prepared separately by flooding week-old plates with sterile nitrogen-free HSM (MARTIN and GOODENOUGH 1975). The cells were suspended at a density of $1-2 \times 10^8$/ml and allowed to sit, illuminated, for 1–2 hr. The mt^+ and mt^- gametes were mixed and allowed to mate for 20 min. The cells were pelleted twice by centrifugation at 4000 $\times$ *g* for 5 min and the supernatant stored in aliquots at −80°C, which also kills any remaining cells.

Construction of plasmid subclones: The λEMBL3 phage used for transformations were part of the chromosome walk on linkage-group VI described in FERRIS and GOODENOUGH (1994) and depicted in Figure 1. Subclones from phage of interest, all derived from the CC-621 (mt^-) genomic library, were made as follows. pNic9.9 contains the 9.9-kb *Sal*I fragment from phage GI7 (Figure 3), extending from 267.3 to 277.2 kb, inserted into the *Sal*I site of pUC13. Note that the *Sal*I site at the left end of the plasmid is from the λEMBL3 polylinker, not from genomic DNA. This polylinker contributes a *Bam*HI site as well. Two kilobases were removed from pNic9.9 by digesting with *Stu*I (which cuts once within the insert) and *Xba*I (one site in the right polylinker), filling in the *Xba*I site with reverse transcriptase and dNTPs, and blunt-end ligating with T4 DNA ligase to create pNic7.9. This eliminated the *Sal*I site in the right polylinker, so pNic7.9 could then be digested with *Sal*I and *Xho*I and religated to eliminate 800 bp on the left end, creating pNic7.1.

An 8.8-kb *Bam*HI/*Sal*I fragment (−139.9 to −148.7 kb) from phage MC8 (Figure 5) was inserted into *Bam*HI/*Sal*I-digested pUC13 to create pThi8.8. Note that the *Sal*I site at the left end of the plasmid is from the λEMBL3 polylinker, not from genomic DNA. This construct was digested with *Stu*I (which cuts once within the insert) and *Sal*I (one site in the left polylinker), and the *Sal*I site was filled in and blunt-end ligated to create pThi7.3. Digestion of pThi8.8 with *Sac*I, which cuts once in the insert and also in the right polylinker, followed by religation of the plasmid, created pThi6.4. Finally, digestion of pThi7.3 with *Sac*I, and religation, created pThi4.9.

A 5.8-kb *Spe*I/*Sal*I fragment (225.9–231.7 kb) from phage CE3 (Figure 3) was inserted into *Xba*I/*Sal*I-digested pUC13 to create pAc5.8. Note that the *Sal*I site at the left end of the plasmid is from the λEMBL3 polylinker, which contributes a *Bam*HI site as well.

Southern analysis: Genomic DNA and Southern blots were prepared as described in FERRIS (1989). Probes were derived as follows. Probe 11 is a 1.2-kb *Sal*I fragment from phage CC3 (derives from mt^-); probe 12 is a 1.0-kb *Hin*dIII/*Eco*RI fragment from phage HJ5 (from mt^+); probe 13 is a 2.1-kb *Xho*I/*Sal*I fragment from phage NK4 (from mt^+). These are the same probes used for RFLP mapping in FERRIS and GOODENOUGH (1994). Probe thiA is a 2.7-kb *Kpn*I/*Apa*I fragment from pThi7.3.

TABLE 1

Chlamydomonas strains

Strain[a]	Genotype
CC-44	*ac-29a mt*$^-$
CC-45	*ac-29 mt*$^+$
CC-85	*nic-7 mt*$^+$
CC-123	*thi-10 mt*$^+$
CC-350	*nic-7 ac-29a mt*$^-$
CC-620	wild type, *mt*$^+$
CC-621	wild type, *mt*$^-$
CC-627	*nic-14 mt*$^+$
CC-631	*nic-17 mt*$^+$
CC-634	*thi-10 pf-14 mt*$^+$
CC-2660	*nic-7 ac-29a kr-u-24-2 mt*$^-$

[a] CC- refers to strains available from the *Chlamydomonas* Genetics Center, Duke University.

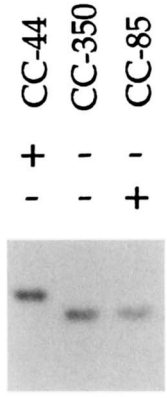

FIGURE 2.—Locating a recombination breakpoint between *nic-7* and *mt*. DNA was prepared from the parent strains, CC-44 and CC-85, and from CC-350, which is recombinant between *mt* and *nic-7*. The relevant genotype of each strain is indicated. The genomic DNA was digested with *Sma*I and a Southern blot hybridized with probe 12 (see Figure 3).

RESULTS

Localization of *nic-7*: A previous report from this laboratory (FERRIS and GOODENOUGH 1994) indicated the location of the *nic-7* gene; presented here are the data that document this localization. The *nic-7* locus maps <0.3 cM centromere-distal of *mt* (SMYTH *et al.* 1975), suggesting that the *nic-7* gene should reside just centromere-distal to the R domain. In their genetic mapping study, SMYTH *et al.* (1975) crossed *nic-7 mt*$^+$ (CC-85) with *ac-29a mt*$^-$ (CC-44). One of the *nic-7 ac-29a mt*$^-$ recombinants was preserved (CC-350), and in fact, all *mt*$^-$ strains carrying *nic-7* derive from this recombinant. The standard *C. reinhardtii* laboratory strains are not isogenic around *mt* (FERRIS and GOODENOUGH 1994), so RFLPs were readily identified to determine the location of the *nic-7*/*mt* recombination breakpoint in CC-350. The Southern blot presented in Figure 2 shows that the RFLP identified by probe 12 (Figure 3) has recombined with *mt* in CC-350 and therefore must be centromere-distal to the recombination breakpoint. Probe 11 (Figure 3) is centromere-proximal to the breakpoint (data not shown), which, therefore, must lie between these two probes, a location that is outside, but close to, the R domain of the *mt* locus, as predicted. The *nic-7* gene must be centromere-distal to this breakpoint, but the linkage disequilibrium around *mt* makes it impossible to predict how many kilobases from the breakpoint *nic-7* might be located.

Transformation was therefore used to pinpoint the *nic-7* gene. The mating type locus chromosome walk comprises overlapping λ phage clones (FERRIS and GOODENOUGH 1994). A 130-kb section of the T domain was divided into five pools. Each pool consisted of purified DNA from four to seven overlapping phage covering ~40 kb. Phage for the pools were chosen to contain large overlaps, thereby reducing the chance that the *nic-7* gene would be split between adjacent phage. Transformation of strain CC-85 to nicotinamide prototrophy (ability to grow in the presence of 3-acetylpyridine) was attempted with five adjoining pools. Transformants were recovered from two adjacent pools. Seven individual phage that span the overlap between those pools were next used (Figure 3), allowing *nic-7* to be localized between the left end of phage GI7 and the right end of phage HV3a.

Because the *nic-7* recipient is *mt*$^+$, the ensuing transformations were made using *mt*$^-$ DNA derived from phage GI7 so that RFLPs could be used to distinguish the endogenous from the transforming copy of the gene. A 9.9-kb *Sal*I fragment from phage GI7 was subcloned to create pNic9.9, which successfully transformed *nic-7*. Results with two smaller subclones, pNic7.9 and pNic7.1 (see MATERIALS AND METHODS), indicated that the insert in pNic7.9 contains the *nic-7*$^+$ gene whereas pNic7.1, which has a much reduced transformation efficiency, probably contains an incomplete copy of *nic-7*$^+$ (Figure 3).

Confirmation of *nic-7* transformation: To verify *bona fide* transformation and not reversion, CC-2660 (*nic-7 ac-29a mt*$^-$) was crossed to two separate Nic$^+$ prototrophs generated by transforming CC-85 with the GI7 phage. In Chlamydomonas, nuclear transformants usually integrate exogenous DNA at a nonhomologous location (DEBUCHY *et al.* 1989; KINDLE *et al.* 1989). Therefore, in a true transformant the functional *nic-7*$^+$ gene will not be linked to *ac-29*$^+$, whereas linkage is expected in a revertant. In both crosses, the *nic-7*$^+$ phenotype segregated 2:2 (implying transformation at a single location), and was indeed unlinked to *ac-29*$^+$ (Table 2).

Further proof of transformation was obtained by ascertaining that transforming DNA was present in the Nic$^+$ cells. Genomic DNA was prepared from several transformants and from a tetrad, digested with *Pst*I, and the resultant Southern blot was hybridized with probe 13 (Figure 4), which is within the *nic-7*$^+$ gene. The probe hybridizes to two *Pst*I fragments (1.9 and 1.3 kb) in the *mt*$^+$ recipient strain, whereas it hybridizes to three *Pst*I fragments (2.0, 1.1 and 0.6 kb) in *mt*$^-$ DNA, from which the transforming DNA derives. Each transformant displays all five fragments (Figure 4). In the tetrad, the introduced *nic-7*$^+$ fragments (t in Figure 4) cosegregate with the prototrophic phenotype but not with *mt* (to which the endogenous *nic-7* is tightly linked).

Two other extant *nic* mutations are also linked to *mt*: *nic-14* and *nic-17*. Both have been assumed to be alleles of *nic-7* (HARRIS 1989), but there are no reports confirming this by complementation tests. Transformation of both *nic-14* and *-17* with GI7 and pNic7.9 yielded prototrophic colonies, and transforming DNA was present in the GI7 transformants (Figure 4). Both mutants also show the low-frequency transformation with pNic7.1. Therefore, they are almost certainly alleles of *nic-7*. The mutations in the three *nic-7* alleles are not the result of

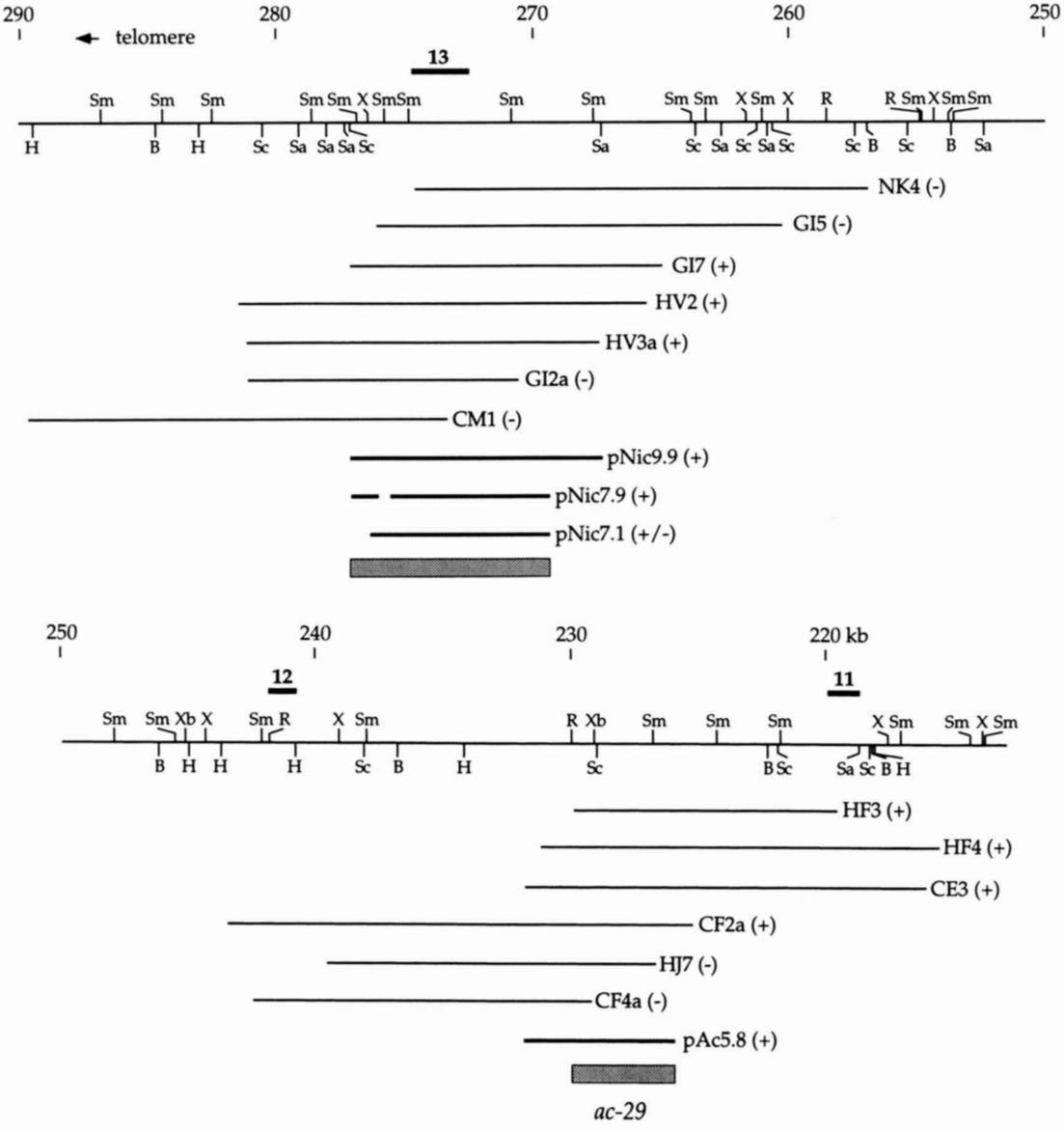

FIGURE 3.—Restriction maps of the *nic-7* and *ac-29* regions in *mt*⁻. A detailed restriction map of the area including *nic-7* and *ac-29* is shown as two contiguous halves, mapped with *Bam*HI (B), *Eco*RI (R), *Hin*dIII (H), *Sma*I (Sm), *Sac*I (Sc), *Sal*I (Sa), *Xba*I (Xb) and *Xho*I (X). The location of the hybridization probes (11, 12, 13) are also shown. Below the map are the locations of the individual phage clones and plasmids that were tested for their ability to rescue *nic-7* or *ac-29*. All plasmids are derived from strain CC-621, as are phage G15, G17, CM1, CE3, CF2a and CF4a. The remaining phage are from CC-620. The (+) or (−) indicates whether the clone was or was not able to rescue the mutation. The plasmid pNic7.1 is marked (+/−) because it consistently gave lower yields of transformants than pNic7.9. Two Nic$^+$ colonies arose in transformations with G15; however, neither of these colonies contained insert DNA and therefore probably represented rare revertants. The shaded boxes represent the maximum size of the *nic-7* and *ac-29* genes.

large (>500 bp) insertions or deletions, as determined by genomic Southern blots (data not shown).

Localization of *thi-10*: Thiamine-requiring auxotrophs map to seven loci, one of which, *thi-10*, maps so close to *mt* that no recombination has ever been reported. The only clue to the location of *thi-10* is a statement in SMYTH *et al.* (1975) that recombination was observed between *nic-7* and *thi-10*, and that *nic-7* was the more centromere-distal marker. None of these recombinants was preserved. Thus, *thi-10* could be anywhere within the region of recombination suppression that is centromere-proximal to *nic-7*, a region of ~800 kb (Figure 1). Phage covering these 800 kb were divided into 19 pools [which exclude the repetitive "swamp" sequences and the multicopy *ezy-1* genes described in FERRIS and GOODENOUGH (1994) and ARMBRUST *et al.* (1993)]. Transformation of *thi-10* was observed using one pool of 13 tested.

Subsequent transformations using individual phage within the area covered by the positive pool (Figure 5) localized the *thi-10* gene between the left end of phage MC8 and the right end of MC3. Because the recipient

TABLE 2

Genetic segregation of the introduced *nic-7*$^+$ gene and *ac-29*

Cross	Tetrads PD:NPD:T	Random progeny[a]
Transformant 1 × CC-2660	2:3:4	Nic$^+$, yellow—16 Nic$^+$, green—8 Nic$^-$, yellow—10 Nic$^-$, green—15
Transformant 2 × CC-2660	0:0:4	Nic$^+$, yellow—6 Nic$^+$, green—6 Nic$^-$, yellow—3 Nic$^-$, green—9

[a] Data from incomplete tetrads; yellow = *ac-29a*, green = *ac-29*$^+$.

thi-10 strain is *mt*$^+$, *thi-10*$^+$ was subcloned from a phage from the *mt*$^-$ library (MC8; see MATERIALS AND METHODS) so that RFLPs could again be used to distinguish the endogenous from the transforming copy of the gene. The longest subclone, pThi8.8, successfully transformed *thi-10* in both the CC-123 and CC-634 strains. Three smaller subclones, pThi7.3, pThi6.4 and pThi4.9 (see MATERIALS AND METHODS), all produced thiamine prototrophs, localizing *thi-10* to a region no larger than 4.9 kb.

Confirmation of *thi-10* transformation: To verify transformation, CC-44 (*ac-29a mt*$^-$) was crossed to a Thi$^+$ strain generated by transforming CC-123 with phage MC8. As expected for a transformant, progeny with the phenotype of the original *thi-10* mutation were recovered, and these were always *mt*$^+$. In addition, Southern blots of genomic DNA purified from six transformants and hybridized with the thiA probe, which is internal to *thi-10*, displayed the 1.6-kb *Pst*I fragment resident in the recipient strain as well as a 2.7-kb fragment unique to the transforming DNA (Figure 6). Transformant 2 was an exception, but because the 2.7-kb *Pst*I fragment is not entirely within the *thi-10*$^+$ gene, this transformant probably contains a truncated copy of the transforming DNA. Genomic Southern blots indicate that the *thi-10* mutation is not the result of a large (>500 bp) insertion or deletion (data not shown).

Methylation of transforming DNA: BLANKENSHIP and KINDLE (1992) have reported that transforming DNA is sometimes methylated, and this was observed in the present study as well. Southern blots were performed using DNA from Nic$^+$ and Thi$^+$ transformants digested with the isoschizomers *Xma*I and *Sma*I, which have different methylation sensitivities (MCCLELLAND *et al.* 1994). The expected pattern of fragments was produced by *Xma*I, but in many transformants *Sma*I failed to generate certain lower molecular weight fragments and instead yielded fragments of higher molecular weight (data not shown), suggesting that some of the *Sma*I sites were methylated. Because the endogenous and transforming genes can be distinguished using *Sma*I RFLPs,

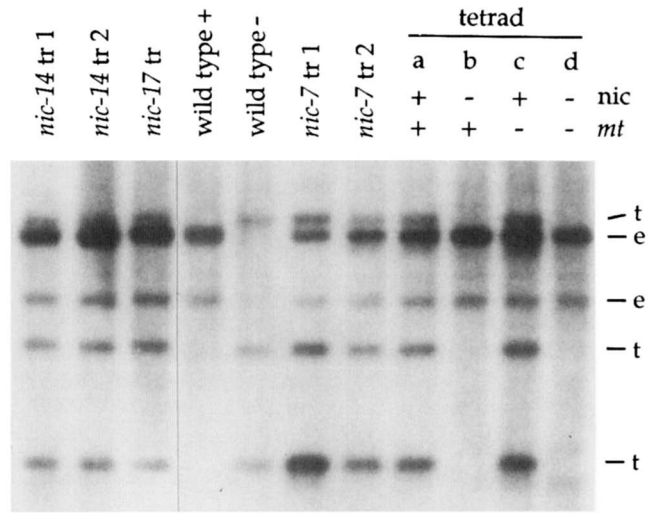

FIGURE 4.—Presence of *nic-7*$^+$ DNA in transformants. A Southern blot of *Pst*I-digested genomic DNA from the indicated strains was hybridized with probe 13, which is internal to the *nic-7* gene (Figure 3). In wild-type *mt*$^+$, and in the three mutant strains *nic-7*, *-14* and *-17* (not shown), there are two hybridizing fragments labeled e (endogenous). Transformation utilized the G17 phage, isolated from the wild-type *mt*$^-$ strain, which has three hybridizing fragments labelled t (transforming). The five transformed strains are designated tr. One tetrad from a cross between *nic-7* transformant 2 and CC-2660 is shown, Note that CC-2660, although *mt*$^-$ derives from the *nic-7 ac-29a* recombinant CC-350, and therefore has the endogenous (e) pattern characteristic of wild-type *mt*$^+$ (not shown). The Nic and *mt* phenotypes of the progeny are indicated.

it was apparent that the methylation was largely (or exclusively) restricted to the transforming DNA.

Localization of *ac-29*: Recombination between *ac-29* and *mt* has never been reported. Based on the one recombinant between *nic-7* and *ac-29* that has been studying in detail (CC-350; see above), it follows that *ac-29* must lie within the region of recombination suppression around *mt* and centromere-proximal to the recombination breakpoint in CC-350. Transformation of strain CC-44 (*ac-29a mt*$^-$) to yield green colonies (see MATERIALS AND METHODS) was attempted using the same 19 phage pools that were used to locate *thi-10*. Transformants were observed using the pool immediately adjacent to the CC-350 breakpoint.

Subsequent transformations using individual phage within the area covered by the positive pool (Figure 3) localized the *ac-29* gene between the left end of phage HF3 and the right end of CF2a. A 5.8-kb fragment from phage CE3 was subcloned into pUC13 to create pAc5.8, which successfully transforms *ac-29a*. This means that the *ac-29* gene is no more than 3.9 kb in size. Strain CC-45, which carries the *ac-29* allele, was also successfully transformed by pAc5.8.

Confirmation of *ac-29* transformation: Transformation was verified genetically for one presumed transformant of CC-44 by crossing it to an *ac-29*$^+$ (green) strain (CC-85). The recovery of yellow progeny demon-

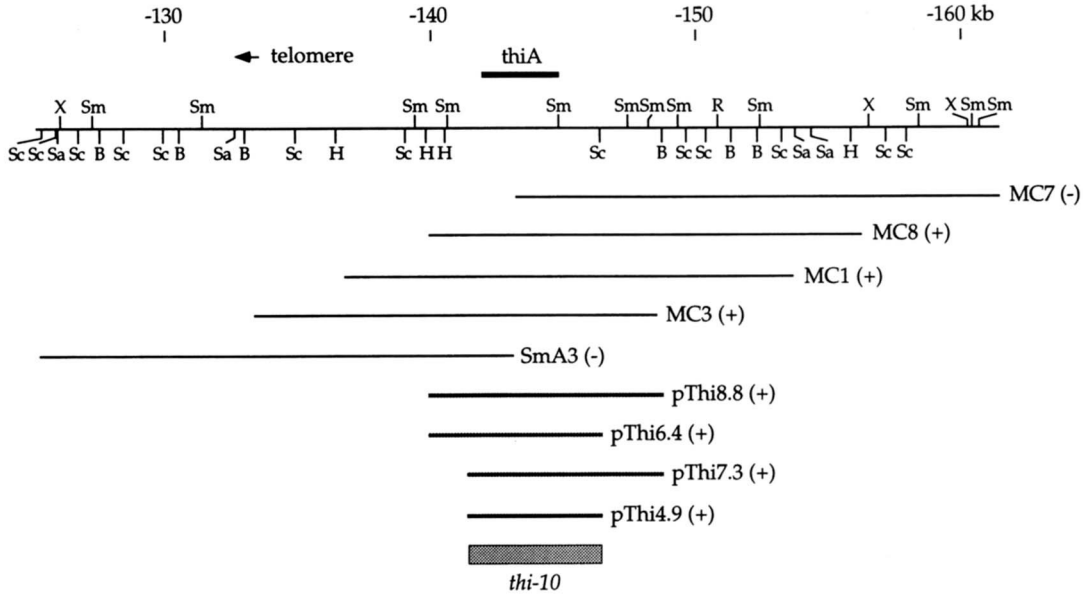

FIGURE 5.—A detailed restriction map of the area around *thi-10*, drawn in the same format as Figure 3. The location of probe thiA is indicated. Below the map are the locations of the individual phage clones and plasmids that were tested for their ability to rescue *thi-10*. All plasmids and phages derived from strain CC-621, except SmA3. The (+) or (−) indicates whether the clone was or was not able to rescue the mutation. The shaded box represents the maximum size of the *thi-10* gene.

strated that the *ac-29a* allele had not reverted. The presence of transforming DNA in putative transformants of CC-44 and CC-45 was demonstrated by Southern blots of genomic DNA (data not shown). However, one of the five presumed transformants of CC-44 contained no transforming DNA and probably represented a revertant of *ac-29a*. Unlike *nic-7* and *thi-10*, it is not possible to use naturally occurring RFLPs within the *ac-29* gene to differentiate endogenous and transforming alleles because the *ac-29* gene resides in a 12-kb region (coordinates 226–238 kb) of the chromosome in which

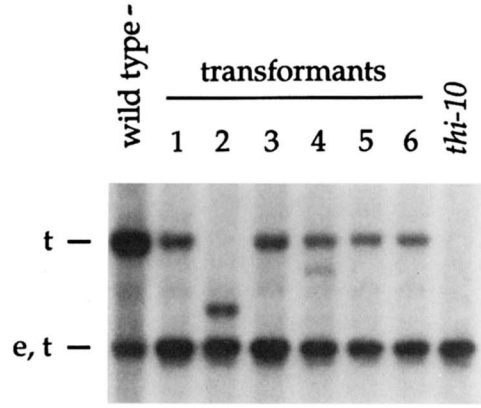

FIGURE 6.—Presence of *thi-10*$^+$ DNA in transformants. A Southern blot of *Pst*I-digested genomic DNA from the indicated strains was hybridized with thiA, which is internal to the *thi-10* gene (Figure 5). The *thi-10 mt*$^+$ strain has a fragment at 1.6 kb (probably as doublet), labeled e (endogenous). The wild-type *mt*$^-$ strain (from which the transforming DNA was derived) shows two fragments of 2.7 and 1.6 kb, labeled t (transforming). Transformants 1–4 were transformed with phage pools that included phage MC1; transformant 5 was transformed with MC3 and transformant 6 with MC8.

no RFLPs are observed between wild-type *mt*$^+$ and *mt*$^-$. Genomic Southern blots indicate that neither the *ac-29* nor the *ac-29a* mutation is the result of a large (>500 bp) insertion or deletion (data not shown).

DISCUSSION

Recombination suppression and the mating-type locus: The three loci described in this report are the ones whose tight linkage to one another and to *mt* led GILLHAM (1969) to postulate recombination suppression around *mt*. The *ac-29* and *thi-10* loci are shown here to be 375 kb apart. Using the approximation of 50 kb/cM in *C. reinhardtii* (FERRIS and GOODENOUGH 1994), the two markers should be separated by a 7–8 cM map distance, yet an unambiguous *ac-29 thi10* recombinant was not recovered in an analysis of 133 tetrads (GILLHAM 1969; SMYTH *et al.* 1975), a clear suppression of recombination. Recombination between *nic-7* and *ac-29* has been observed, but only in three out of >1000 tetrads (EBERSOLD *et al.* 1962; GILLHAM 1969; SMYTH *et al.* 1975). Because *nic-7* and *ac-29* are shown here to be only 40 kb apart, they would not be expected to recombine readily in any case, but their recombination is so infrequent as to suggest that they are under recombinational suppression as well.

The *thi-10* and *ac-29* genes are found on either side of the R domain, in regions where no recombination breakpoints between *mt* and flanking markers have been detected (FERRIS and GOODENOUGH 1994). Besides failing to recombine with each other, recombination between *thi-10* and *mt*, or between *ac-29* and *mt* has not been observed (EBERSOLD *et al.* 1962). This is consistent with the fact that *mt*-linked genes involved in

generating a mating phenotype have thus far been found to lie within the R domain itself (P. J. FERRIS and U. W. GOODENOUGH, unpublished data). Because neither *thi-10,* nor *ac-29,* nor *nic-7* localizes within the R domain, this suggests that the R domain may prove to be composed exclusively of genes with mating functions. It follows that other *mt*-linked housekeeping genes, such as *mbo-1, fa-1* and *cyt-1* (FERRIS and GOODENOUGH 1994), are also predicted to localize within the DNA flanking the R domain.

The *nic-7* gene: The cloning of the *nic-7* gene provides a useful new selectable marker for *C. reinhardtii* transformation, one that has already been used successfully in this laboratory. Untransformed *nic-7* cells die back completely on 3-acetylpyridine within a few days, and the tight linkage of *nic-7* to *mt* can be useful in subsequent crosses because a progeny's mating type indicates whether it carries the mutant or wild-type allele at the *nic-7* locus. The *nic-7* gene may also potentially be used to identify a transformation recipient for *C. eugametos* since nicotinamide-requiring mutants have been identified in that species (HARRIS 1989).

Two other nicotinamide-requiring mutations, *nic-14* and *nic-17,* are linked to mating type, and both are rescued by transformation with the pNic7.9 plasmid. These could be mutations in a second gene involved in nicotinamide biosynthesis and closely linked to *nic-7,* but the fact that the smallest fragment (7.9 kb) that will rescue *nic-7* also rescues *nic-14* and *nic-17* suggests they are all within the same gene.

pNic7.1 reproducibly gave much lower yields of transformants than pNic7.9. This is most readily interpreted to mean that the 800 bp removed from pNic7.9 to create pNic7.1 contains sequences necessary for efficient expression of *nic-7.* Transformation by pNic7.1 may only be possible if the plasmid integrates into certain locations, or if it is present in multiple copies. Consistent with this, two (of three) pNic7.1 transformants analyzed appear to contain multiple copies of *nic-7*$^+$ sequences (data not shown).

The *thi-10* gene: Cloning *thi-10* also provides a potential new selectable marker, although untransformed *thi-10* cells do not die back as readily as do other auxotrophs currently being used. *Thi-10* does have the advantage of being transformable by a relatively small segment of DNA—4.9 kb, as opposed to 7.8 kb for *arg-7* (DEBUCHY *et al.* 1989), 7.9 kb for *nic-7* (present study), or 14.5 kb for *nit-1* (FERNANDEZ *et al.* 1989).

The *ac-29* gene: Transformation of *ac-29* by the serial dilution technique is slow and tedious. Only one potential transformant is obtained per tube transferred, since all green cells must be assumed to be clonal, and that putative transformant may actually be a revertant. *Ac-29* will therefore not be very practical as a cotransformation selectable marker.

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