LINKAGE OF MUTATIONS AFFECTING MINUS FLAGELLAR MEMBRANE AGGLUTINABILITY TO THE mt^- MATING-TYPE LOCUS OF CHLAMYDOMONAS

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ABSTRACT

Two independently isolated mutant strains, *imp-10* and *imp-12*, were obtained by UV irradiation of wild-type mating-type minus (wt^-). Each fails to agglutinate sexually with gametes of either mating type, but mating and zygote formation can be elicited by agglutinating either strain to wt^+ gametes by means of anti-flagellar antiserum. Tetrad analysis of the resultant zygotes shows that both *imp-10* and *imp-12* are very closely linked to mt^-, with no recombinants observed. Diploid strains constructed between *imp-10* or *imp-12* and wt^+ gametes are wt^-, that is, they agglutinate and fuse like normal minus cells. Tetrad analysis of triploids from *imp-10* diploid $\times$ wt^+ haploid crosses shows that only *imp-10* and wt^+ products are recovered. A model is proposed to account for these results.

THE mating-type locus of *Chlamydomonas reinhardi*, located in the left arm of linkage-group VI (EBERSOLD *et al.* 1962; see Figure 1), segregates as a pair of Mendelian alleles, two meiotic products being mt^+ and two mt^- (SMITH and REGNERY 1950). To date, three markers have been described that exhibit close linkage to *mt* (Figure 1) and that relate to known sexual phenotypes: the *mat–1* and *mat–2* mutations are linked, respectively, to mt^- and mt^+ and affect the *mt*–regulated transmission of chloroplast genes (SAGER and RAMANIS 1974), while the *imp–1* mutation maps to the mt^+ locus and affects gametic cell fusion (GOODENOUGH, HWANG and MARTIN 1976; GOODENOUGH and WEISS 1975).

We report here the isolation of two mutant strains, *imp–10* and *imp–12*, from irradiated wild-type minus (wt^-) cultures. Both have lost minus flagellar agglutinability and will mate with wild-type plus (wt^+) gametes only in the presence of anti-flagellar antisera (GOODENOUGH, HWANG and WARREN 1978; GOODENOUGH and JURIVICH 1978). Each mutation is shown to be tightly linked to mt^-; thus, they represent the first mating-related markers for this locus.

We have used the *imp–10* and *imp–12* strains to probe the behavior of the mating-type loci in diploid strains. Knowing that wt^-/wt^+ diploids are phenotypically minus, we expected that *imp–10* mt^-/wt^+ and *imp–12* mt^-/wt^+ diploids would be nonagglutinable or, possibly, would agglutinate like plus. Sur-

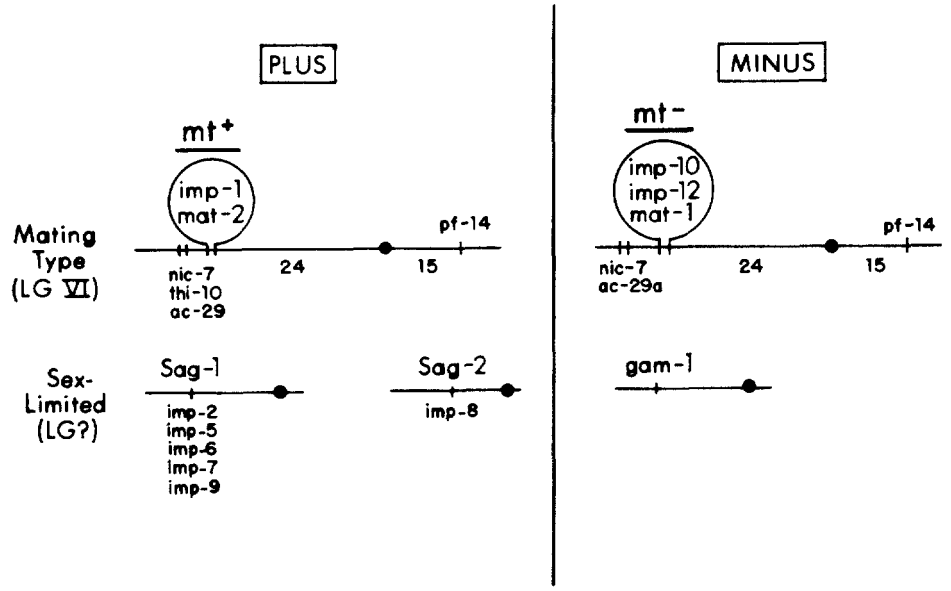

FIGURE 1.—Identified gene loci involved with the Chlamydomonas mating reaction. The *sag-1* and *sag-2* loci control mt^+ agglutinability; the *imp-10* and *imp-12* loci are shown in this paper to control mt^- agglutinability; the *gam-1* locus controls sexual signaling in mt^-; the *imp-1* locus controls gametic cell fusion in mt^+; and the *mat-1* and *mat-2* loci control chloroplast gene transmission. Genetic analysis of the *imp* mutations is described in GOODENOUGH, HWANG and MARTIN 1976, and GOODENOUGH, HWANG and WARREN 1978. The *imp-9* mutation, isolated by C. FOREST, was found by A. J. WARREN to be closely linked to other *sag-1* markers (unpublished data from this laboratory). The *gam-1* mutation (FOREST and TOGASAKI 1975; FOREST, GOODENOUGH and GOODENOUGH 1978) is not linked to the *sag* loci (A. J. WARREN, unpublished data). Information on *mat-1* and *mat-2* is found in SAGER and RAMANIS (1974), *imp-1* in GOODENOUGH and WEISS (1975) and the other *imp* mutations in BERGMAN *et al.* (1975).

prisingly, all such diploids agglutinate like minus, indicating that the wt^+ genome, or some feature of the diploid state, provides the requisite information for minus agglutinability. To ask whether this "correction" is heritable, one of the diploids was crossed with wt^+ and the triploids subjected to tetrad analysis. Only wt^+ and *imp* products were recovered, indicating that the wt^- phenotype of the diploid is not transmitted. A model is proposed to account for these observations.

MATERIALS AND METHODS

Strains, growth conditions, mutagenesis and genetic analysis: The 137c-H wild-type strains in our collection carry lethal factors that impede genetic analysis (GOODENOUGH, HWANG and MARTIN 1976). Clones with reduced meiotic lethality and high mating efficiency were therefore selected from 137c-H in 1976; these are called 137c-R_3 (mt^+) and 137c-no (mt^-) (designated CC-396 and CC-397, respectively, in the Chlamydomonas Genetics Center, Duke University, Durham, N.C.), and are designated simply wt^+ and wt^- in all experiments reported here. Recent genetic manipulations reveal that lethality persists in $wt^+ \times wt^-$ crosses,

but, as found for the parental strains (GOODENOUGH, HWANG and MARTIN 1976), particular markers are not selectively affected. Lethal-free strains (CC-124 and CC-125) of 137c, it should be noted, are now available from the Chlamydomonas Genetics Center, although their mating efficiencies have not been reported. The *CC-125* strain was utilized in the vegetative diploid crosses reported here. The mutant strains *arg-2* (EVERSOLE 1956; also called *arg-7-8*, LOPPES and MATAGNE 1972), *arg-7* (GILLHAM 1969) and *pf-14* (LUCK *et al.* 1977; WITMAN, PLUMMER and SANDER 1978) were also used in various crosses; their Stock Center designations are CC-48, CC-50 and CC-930, respectively.

Cell culture, mutagenesis, screening and genetic analysis of mating mutants were performed as previously described (GOODENOUGH, HWANG and MARTIN 1976; GOODENOUGH, HWANG and WARREN 1978) except that the wt^- strain was UV irradiated. and an *arg-2* mt^+ strain was used in selecting for nonmating mt^- mutants by the pellicle exclusion screen. The Stock Center designations for the mutants reported here are CC-1147 (*imp-10*) and CC-1149 (*imp-12*).

Vegetative diploids were selected as complementary prototrophic diploids (EBERSOLE 1967; LOPPOS and MATAGNE 1972) following antibody-mediated crosses (GOODENOUGH, HWANG and WARREN 1978; GOODENOUGH and JURIVICH 1978) of a constructed mt^- *imp-10 arg-2* strain with an mt^+ *arg-7* strain. The diploid $\times$ haploid cross was performed with CC-125 mt^+ haploids; the resultant zygotes were allowed to germinate and grow on arginine-supplemented medium.

Agglutinin analysis: Flagella are prepared by the pH shock method of WITMAN *et al.* (1972) with 10 mM PIPES pH 7.0 used in place of Tris. Flagella are resuspended in 5% sucrose, 10mM PIPES pH 7.0, at a protein concentration of 2 mg/ml and Aproteinin (Sigma Chemical Company) added to 10% w/v. To this mix is added an equal volume of 60 mM octylglucoside, 10^{-4} M EGTA and 10^{-4} M dithiothreotol in 10 mM PIPES pH 7.0. The flagella are extracted for 45 min on ice; axonemes and mastigonemes are then removed by centrifugation at 100,000g for 1 hour. The supernatant is dialyzed overnight against 5 1 distilled water to remove detergent, centrifuged at 100,000g for 1 hr to remove particulate matter, concentrated by freeze-drying, and resuspended in a small volume of distilled water. This fraction, referred to as dialyzed octylglucoside supernatant (DOGS), is serially diluted and 1 μl aliquots are spotted on glass slides and allowed to dry at room temperature to form films. Gametic tester cells specifically adhere by their flagellar tips to films derived from complementary wt gametes. Detailed biochemical analyses of DOGS will be reported elsewhere (ADAIR *et al.* 1982).

RESULTS

Phenotypes of imp–10 *and* imp–12: The *imp–10* and *imp–12* mutant strains are indistinguishable from their wt^- parents in growth rates, motility and morphology. When starved of nitrogen, moreover, the mutant gametes will agglutinate to one another by their flagellar tips when presented with an antiserum directed against the flagellar surface; (GOODENOUGH and JURIVICH 1978); such "tipping" of antibody is a differentiated gametic trait (GOODENOUGH and JURIVICH 1978). Both mutant strains fail, however, to agglutinate sexually with either wt^+ or wt^- gametes.

To determine whether *imp* flagella carry low levels of agglutinin—that is, whether the mutations cause quantitative rather than qualitative changes in adhesiveness—we prepared dialyzed detergent extracts of their flagellar membranes (see MATERIALS AND METHODS) and compared their agglutinin titers with wild-type extracts. Whereas the wt^- extracts agglutinate wt^+ tester gametes up to 11 serial dilutions, giving a specific activity of 2×10^6 units/mg

protein, equivalent mutant extracts are inactive even without dilution, giving a specific activity of <400 units/mg.

If either *imp–10* or *imp–12* gametes are mixed with wt^+ gametes in the presence of anti-flagella antiserum, the resultant flagellar agglutinations activate all the subsequent steps in the mating reaction, and efficient zygotic cell fusion occurs, much as we have previously reported for nonadhesive mt^+ mutants (GOODENOUGH, HWANG and WARREN 1978; GOODENOUGH and JURIVICH 1978). Therefore, the *imp–10* and *imp–12* mutations each appear to affect a single gamete trait and do not produce multiple pleiotropic effects on gametogenesis.

Genetics of imp–10 *and* imp–12: When antibody-mated zygotes are allowed to undergo meiosis and are subjected to tetrad analysis, the data summarized in Table 1 are obtained. In *imp–10* $mt^- \times + mt^+$ crosses (Table 1, Cross 1), the tetrads are all parental ditypes containing wt^+ and nonagglutinating *imp* products in a 2:2 ratio: no recombinant wt^- gametes emerge from the cross. A similar absence of recombination is found in *imp–12* $mt^- \times + mt^+$ crosses (Table 1, Cross 2). To demonstrate that the *imp* mutations do not act as suppressors of recombination, the *imp–10* mutant was antibody-mated to *pf–14* (*p*aralyzed *f*lagella), a locus residing in the right arm of LG VI approximately 40 map units from the *mt* locus (Figure 1). As summarized in Table 1, Cross 3, the expected level of recombination was observed between *mt* and *pf* in the presence of the *imp* mutation, ruling out a generalized suppression of crossing over.

To determine whether we could detect any very rare recombination between the *imp* and mt^- loci, the following manipulations were performed. Each *imp* strain was antibody-mated with wt^+. The resultant mature zygotes were chloroformed to kill unmated cells and were allowed to mass-germinate, yielding about 100 zygotic colonies per cross. Each of the $1000 \times 4 = 4000$ meiotic products was allowed to undergo the approximately 10 rounds of mitosis that precede the final gametogenic mitosis; this means that if any of the meiotic products were a wt^- recombinant, it would have been amplified into a clone of about 1000 gametes, so that at least one recombinant would be likely to be detected in the subsequent screen. The total collection of gametes was then suspended in liquid NF medium, this time without antibody, the reasoning being that any wt^- recombinant gamete would have ample opportunity to agglutinate and fuse with one of the many wt^+ gametes present in the suspension. Finally, this mating mixture was plated and subjected to a second round of chloroforming and mass-germination. This time, the resultant plates were found

TABLE 1

Tetrad analysis of crosses involving imp-10 *and* imp-12

Cross	Parents	PD	T	NPD
1	$+ mt^+ \times$ *imp-10* mt^-	35	0	0
2	$+ mt^+ \times$ *imp-12* mt^-	14	0	0
3	$+$ *pf-14* $mt^+ \times$ *imp-10* $+ mt^-$	1	14	0

to contain *no* zygotic colonies, indicating that no wt$^-$ clones had been generated. In other words, there is no evidence for recombination between the *imp* loci and *mt*$^-$ in roughly 10^3 meioses.

Behavior of imp–10 *and* imp–12 *in diploids*: Since the *mt*$^-$ locus is dominant to *mt*$^+$ in diploid strains of *C. reinhardi* (EBERSOLD 1967), we constructed + *mt*$^+$/*imp–10 mt*$^-$ and + *mt*$^+$/*imp–12 mt*$^-$ diploid strains to learn whether the *imp–10* and *imp–12* mutations are also dominant. A total of 48 prototrophic colonies was recovered from + *mt*$^+$ *arg–2* × *imp–10 mt*$^-$ *arg–7*; 16 colonies were analyzed from the analogous *imp–12* cross. When allowed to differentiate into gametes, all 64 strains proved to agglutinate and fuse like wt$^-$: none exhibited any *mt*$^+$ or *imp* traits. In other words, not only are the *imp* mutations recessive, but information for the synthesis of normal *mt*$^-$ agglutinin is somehow provided by the wt$^+$ parent and/or allowed to be expressed in the diploid state.

To ask whether these prototrophic strains are in fact diploid and whether they carry both parental markers, one of the 48 *imp–10* diploids was crossed to wt$^+$. The resultant triploid zygotes germinated well (>50%), but yielded few viable products, as observed previously (GILLHAM 1969). Table 2 summarizes the phenotypes of these products: it is seen that the *imp–10* and wt$^+$ markers are recovered; whereas, the wt$^-$ phenotype of the diploid parent is not expressed in any of the progeny.

DISCUSSION

The *imp–10* and *imp–12* strains of *C. reinhardi* are the first reported mutations affecting *mt*$^-$ agglutinability. The fact that concentrated extracts of mutant flagellar membranes are also inactive in a sensitive agglutination bioassay indicates that each mutation affects either the synthesis of the adhesive molecules or their ability to be included in flagellar membranes. Further analysis of the molecular nature of the mutations awaits a definitive biochemical identification of the wt agglutinins, which, to date, has not been achieved (ADAIR *et al.* 1982).

The *imp–10* and *imp–12* mutations were independently derived and both are shown to be very closely linked to the *mt*$^-$ locus. Whether they are alleles or

TABLE 2

Phenotypes of meiotic products from a cross between + mt+ arg-7/imp-10 mt- arg-2 *diploids and* + mt+ *haploids*

Zygote	# Viable meiotic products	Phenotypes
1	4	2 *imp*, 2 wt$^+$
2	3	2 *imp*, 1 wt$^+$
3	2	2 wt
4	2	1 *imp*, 1 wt$^+$
5	2	2 *imp*

Plus 10 zygotes with one survivor each: 6 were *mt*+ and 4 were *imp*.

affect two closely linked genes can be assessed only by artificial cell fusions using polyethylene glycol (MATAGNE, DELTOUR and LEDOUX 1979); construction of appropriate strains for such analyses is currently underway.

Mass matings show that the *imp–10* mutation fails to become separated from the mt^- locus in at least 1000 meioses, giving an apparent recombination frequency of $<10^{-3}$. The documented suppression of crossing over in the left arm of LG VI (GILLHAM 1969), however, means that recombinational analyses of this sort cannot be used to *prove* that either mutation lies within mt^- itself. Since both mutations affect a specific gametic trait, however, we consider the hypothesis highly probable.

The close linkage of *imp–10* and *imp–12* to mt^- stands in sharp contrast to the properties of mutations affecting the plus flagellar agglutinin. We have previously shown (GOODENOUGH, HWANG and WARREN 1978; see also Figure 1) that mutations of two gene loci, *sag–1* and *sag–2*, affect mt^+ agglutinability and are expressed only in mt^+ cells, but are not linked to the mt^+ locus. We have recently demonstrated (ADAIR *et al.* 1982) that none of the six *sag* strains carries active agglutinin in DOGS extracts of their flagellar membranes; they therefore also qualify as affected in structural or "flagellar-insertion" genes. Thus, a marked asymmetry exists in the genetic specification of the plus and minus agglutinin systems even though, in our biochemical experiments, we find that the partially purified agglutinins of the two mating types have similar properties: both appear to be very large, glycosylated, extrinsic membrane proteins that are present in very few copies per flagellum (ADAIR *et al.* 1982).

A number of models can be proposed to explain why diploids constructed between wt^+ and either *imp–10* or *imp–12* exhibit normal minus agglutinability. The most straightforward, however, is to propose that the two *imp* mutations mark a locus that we can designate *sad –1* (*s*exual *ad*hesion), which is linked to *mt* but sex-limited in its expression to mt^- cells. By this model, a wt^+ cell would possess a normal copy of *sad–1* linked to its mt^+ locus; this would not be expressed against its mt^+ haploid background, but would be expressed in the presence of the dominant mt^- locus in diploid strains. The major genetic difference between *sad–1* and the other sex-limited loci identified in *C. reinhardi* (FOREST and TOGASAKI 1975; GOODENOUGH, HWANG and WARREN 1978), therefore, would be its linkage to *mt*.

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