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Genetics

Loci affecting flagellar assembly and function map to an unusual linkage group in *Chlamydomonas reinhardtii*

(basal bodies/*uni* linkage group/circular map)

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ABSTRACT **The *uni*1 mutant of *Chlamydomonas reinhardtii* lacks one of the paired flagella seen in wild-type cells. The missing flagellum is cis to the eye spot and at this site the basal body is incomplete, lacking a transition zone. Together with nine other loci affecting flagellar assembly and two loci affecting flagellar function *uni*1 defines an ≈100-centimorgan linkage group with a circular map. Measurements of gene–centromere distances on the *uni* linkage group are consistent with the ordering of loci determined by recombination analysis and place the centromere near *pf*29, a motility-defective mutant. In well-synchronized meiotic cycles, recombination frequencies between loci on the *uni* linkage group but not loci on other linkage groups show striking temperature sensitivity during a 1- to 2-hr period 5 days before meiosis.**

In the unicellular alga *Chlamydomonas reinhardtii*, as in all eukaryotic cells, flagellar shafts are assembled onto basal bodies in the flagellar apparatus (1). Mutants at the *uni* locus of *C. reinhardtii* show failure of assembly of one of the paired flagella because the mutation prevents complete development of one of the basal bodies (2). Using the eye spot (located on a lateral surface of the cell) as an anatomical marker we found that >95% of mutant uniflagellate cells lacked the flagellum cis to that structure. Furthermore, these mutants failed to map to any of the 18 known linkage groups in *C. reinhardtii* (2) (unpublished data).

We report here that the *uni* locus along with nine other loci involved in flagellar assembly and two loci affecting flagellar function define a new linkage group with a circular map: the *uni* linkage group (ULG).

EXPERIMENTAL PROCEDURES

Mutant Strains. Mutant *pf*27 and all ULG mutants except for *pf*7, *pf*8, and *pf*10 were isolated in this laboratory. The last three mutants and all other strains not isolated here were obtained from the *Chlamydomonas* Stock Center at Duke University (Durham, NC). Mutants isolated in this laboratory were crossed to wild type, and after cloning, F_1 or F_2 daughters were used for genetic analysis.

Tetrad and zygote clone analyses were performed at 21°C by using standard techniques (3). Map distance [centimorgans (cM)] was computed by using the equation (4) $[(6NPD + T)/(PD + NPD + T)] \times 50$, where *NPD* equals number of nonparental ditype tetrads, *PD* equals parental ditype tetrads, and *T* equals tetratype tetrads. Gametic cells were produced by growth on low-sulfate agar medium followed by a 4-hr culture in nitrogen-free liquid medium (5). Under these conditions wild-type cells generate flagella within 30 min after transfer to liquid.

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RESULTS

Mutant Phenotypes. Except for *uni* each locus of the ULG is at present defined by a single mutant. The mutants and the mutagens used for their induction are listed in Table 1.

Mutants *fla*9 through *fla*13 lack flagella when cultured at 32°C but differ in their capacity to generate flagella at 21°C (see *Experimental Procedures*). Rapid generation of flagella is observed in *fla*9 and *fla*10; the other mutants produce flagella in ≈2 hr (*fla*12 and *fla*13) or ≈24 hr (*fla*11). Shifting flagellated cultures from 21 to 32°C results in rapid resorption of flagella for *fla*10 (complete in 4 hr) and in slow resorption of flagella for the other *fla* mutants (complete in ≈24 hr).

The mutant strain *pf*7 has short flagellar stubs; *pf*8 totally lacks flagella. Mutants *pf*10 and *pf*29 have full-length flagella but the mutant cells exhibit abnormal motility. Cells of *pf*10 show little linear movement and tend to travel in small irregular circles; in liquid culture they form a tight pellet. Cells of *pf*29 move slowly in jerky linear paths; in liquid culture they form a loose pellet with a zone of swimming cells above.

Two mutants, *sun*1 and *enh*1, lack independent phenotypes but modify differently the *uni*1 phenotype so that double mutants can be scored. In exponential cultures of *uni*1, 90% of cells are uniflagellate, <5% have no flagella, and the remainder are biflagellate (2). In the *uni*1 *sun*1 double mutant the population of biflagellate cells is increased to 70–80%, whereas in the *uni*1 *enh*1 double mutant 70–80% of the cells lack flagella.

Genetic Map of the ULG. For each mutant Mendelian 2:2 segregation was established by crosses to wild-type and to ULG mutants with independently scorable phenotypes. In crosses between *fla* mutants and between ULG *pf* mutants, the double mutant recombinants frequently lacked flagella; tetrads showing 3:1 segregation of nonswimming cells were assumed to be T tetrads, and tetrads showing 2:2 segregation were assumed to be NPDs.

Genetic analysis for the ULG gave an internally consistent, 100-cM circular map that is presented in linear form, opened at *uni* (Fig. 1).

Not shown on the map are linkage relationships for three other mutants: *enh*1 was found to be 3 cM to the left or right of *uni*1 (35 tetrads); *pf*7 was 4 cM (65) to the left of *uni*1, 17 cM (84) from *fla*9, and 26 cM (72) from *fla*10 (numbers in parentheses include data from tetrad and zygote clonal analysis). We were unable to obtain any crosses with *pf*8, but it is included on the ULG based on the data of McVittie, who estimated linkage to *pf*7 to be ≈5 cM (10).

Gene–Centromere Distances on the ULG. Exchange frequencies between mutants at nine loci on the ULG and its centromere were estimated by pairwise crosses to *ac*17. The *ac*17 marker is closely linked to the centromere of linkage group III (11). Observations of second-division segregation

Abbreviations: ULG, *uni* linkage group; NPD, nonparental ditype; PD, parental ditype; T, tetratype; cM, centimorgan(s).

Table 1. Loci of the ULG

Mutant	Mutagen	Ref.
*uni*1	ICR 191	6
*sun*1	UV	5
*enh*1	ICR 191	6
*fla*9	MNNG	7
*fla*10	MeMes	8
*fla*11	MNNG	7
*fla*12	MNNG	7
*fla*13	MNNG	7
*pf*7	UV	9
*pf*8	UV	9
*pf*10	UV	9
*pf*29	ICR 191	6

Mutagenic agents: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MeMes, methyl methanesulfonate.

frequencies from these crosses gave direct estimates of gene–centromere distances on the ULG (4).

The results of this analysis, shown in Fig. 2, are consistent with the order of loci on the genetic map; they place the centromere at a position close to *pf*29. For *uni*1, *pf*10, and *pf*29 confirmatory data were obtained by using *pf*27, a marker linked to the centromere of linkage group XII (6).

T frequencies in the region *fla*11 to *fla*13 exceed 0.667, a theoretical limit expected because of the effect of multiple exchanges likely to occur between loci distant from the ULG centromere and the centromere (4). Positive chiasma interference or chromatid interference could be a cause of this excess of T tetrads (12).

We have not yet obtained adequate numbers of tetrads from 3-factor crosses in the *uni*1–*fla*9–*fla*10 intervals to determine the possible role of chiasma or chromatid interference. However, Papazian (13) has derived the following equation for assessing interference from 2-factor cross data. $NPD = \frac{1}{2}[1 - T - (1 - 3T/2)^{2/3}]$. This equation predicts the expected frequency of NPD tetrads from the frequency of T tetrads in the absence of chiasma or chromatid interference. Using Papazian's equation, 2-factor cross data from the experiments of Fig. 1 have been analyzed (Table 2).

For intervals distant from the centromere, *uni*1–*fla*9, *fla*10–*pf*10, and *sun*1–*uni*1, the disparity between observed and expected NPD tetrads suggests that there is positive interference, but the observation does not allow distinction between positive chiasma interference and chromatid interference. For the remaining intervals that cross the region of the centromere, NPD tetrads appear to be equal to or exceed expectation.

Recombination Frequencies Between Loci on the ULG Are Temperature Sensitive. In the protocol used for the sexual cycle of *C. reinhardtii* the events leading up to and including meiosis can be tightly synchronized (3). Starting with the mixing of populations of mature gametes of opposite mating type the protocol can be divided into three phases: I, 18 hr in light; II, 5 days in darkness; and, finally, III, 15–24 hr in light. Meiosis begins after 10 hr in phase III (14).

We observed that alterations of temperature in phase II but not in phase I or III significantly altered recombination frequency for loci on the ULG. A representative experiment is shown in Fig. 3 for a 5-factor cross: *fla*9 *msr UNI*1 *SUN*1 *ERY*3 × *FLA*9 *MSR uni*1 *sun*1 *ery*3. The mutants *msr* (methionine sulfoximine resistance) and *ery*3 (erythromycin resistance) are linked on the right arm of linkage group I (11). In the experiment, phases I and III were carried out at 21°C and phase II was carried out at the temperatures shown on the abscissa. Phase II temperature shifts between 17 and 32°C led to increased recombination for the two intervals measured on the ULG but had no effect on the linkage group I markers. Additional 2-factor cross data (Table 3) show phase II temperature-dependent increase in recombination for other intervals spanning the ULG. On the other hand, map intervals between markers on linkage group IX (*pf*16 and *pf*13) and on II (*pf*12 and *pf*18) show no temperature dependence.

To determine whether phase II temperature changes brought about permanent alterations in the *uni* chromosome, we studied progeny from *uni*1 *FLA*9 × *UNI*1 *fla*9 crosses made at 17, 21, and 32°C. From each temperature a T tetrad was selected and the *UNI*1 *FLA*9 segregant was crossed to its *uni*1 *fla*9 sister. F_1 zygotes from these crosses were analyzed at three phase II temperatures. As shown in Table 4 the temperature of origin of segregants had no effect on the subsequent recombination behavior of the F_1 zygotes. Furthermore, recombination in each F_1 cross responded as expected to shifts in phase II temperature. The effect of elevation of temperature in phase II is to increase the frequency of recombination without producing a heritable change in the ULG.

By studying the effect of temperature shifts at the beginning of phase II, when zygotes were shifted from light to dark, we observed that the critical period for temperature elevation to increase recombination was limited to the first 1–2 hr.

In the first series of experiments, Table 5 (column 2), zygote products of *uni*1 *FLA*9 × *UNI*1 *fla*9 crosses were held at 17°C for phase I and transferred to dark at the same temperature, and after intervals varying from 0.5 hr to 24 hr they were shifted to 32°C for the duration of phase II. Phase III was carried out at 21°C. When the results of delay in temperature shifts are compared with the result of a control cross in which phase II was maintained at 17°C, it is apparent

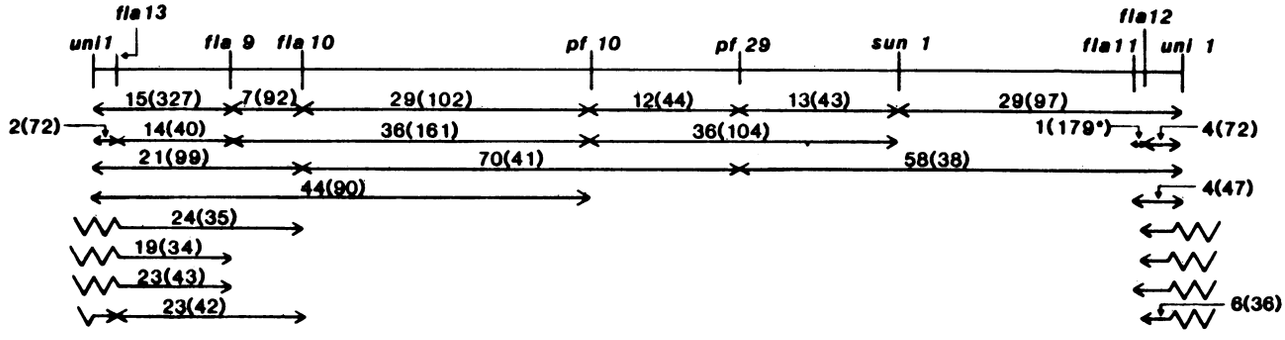

FIG. 1. Genetic map of the ULG. Intervals between arrowheads designate the map distance (cM) determined by tetrad analyses of pairwise crosses, except for *fla*11 *fla*12, where zygote clones were analyzed. Numbers in parentheses indicate total number of tetrads or zygote clones (179*) analyzed. The circular map is presented in a linear form opened at *uni*1. For analysis of the *sun*1–*pf*29 and *sun*1–*pf*10 intervals, *uni*1 was present in both parents.

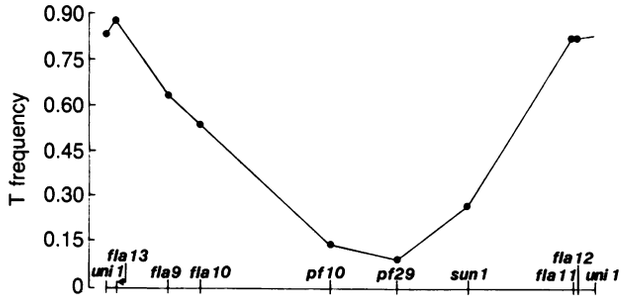

FIG. 2. T frequencies in crosses with the centromere-linked marker *ac*17. The abscissa shows map location of ULG loci derived from Fig. 1. The ordinate shows T frequencies for *ac*17 in pairwise crosses with the loci indicated. Each point is based on analysis of 39–114 tetrads. In each analysis the frequency of PD to NPD tetrads was approximately equal.

that when the temperature shift to 32°C is delayed for 1–2 hr the effect of temperature elevation is no longer seen.

In the second series of experiments, Table 5 (column 3), *uni*1 *FLA*9 × *UNI*1 *fla*9 zygotes were maintained at 17°C for phase I, and the temperature was equilibrated by transferring the zygotes to 32°C for 30 min. The zygotes were placed in the dark, held for durations of 0.5–24 hr at 32°C, and transferred back to 17°C for the remainder of phase II. Phase III was carried out at 21°C. When these results are compared with the control values for zygotes held constantly at 32°C throughout phase II, it is apparent that an exposure to 32°C for the first hour immediately after shift to dark is adequate to give a maximum effect on recombination.

DISCUSSION

The ULG is different from the other 18 linkage groups of *C. reinhardtii* in three respects: (*i*) recombination analysis gives a circular map; (*ii*) recombination frequencies for loci on the ULG but not other linkage groups are sensitive to temperature at a time 5 days before meiosis; and (*iii*) the extensive clustering of related mutant phenotypes for loci on the ULG is not seen on other linkage groups (11). However, the failure to find mutants other than those affecting flagellar functions on the ULG needs the test of time.

Temperature effects on recombination have been observed in a variety of organisms; in almost every case the sensitive period occurs during meiosis (reviewed in ref. 15). For the ULG, temperature shifts affect recombination during a critical 1- to 2-hr period 5 days before meiosis. The effect appears to be on genetic exchange and is not heritable, and the resulting tetrads are mainly T tetrads. If in gametes the ULG chromosome is single copy it follows that replication must occur before or in association with crossing-over. Therefore, the possibility exists that replication and exchange on the ULG occurs 5 days before meiosis during the first 1–2 hr in the dark phase of zygotic maturation.

A model consistent with the genetic data is that the ULG at the time of crossing-over is a ring chromosome with a single centromere and two chromatids. A problem with this model is that single crossovers, the most likely exchange to generate the predominant T tetrads we observed, would also generate dicentric rings (16). For consistent patterns of 2:2 segregation secondary exchanges elsewhere involving the same strands might be required to resolve these dicentric rings.

Stahl (17) has pointed out that a linear chromosome may give a circular map when crossovers, occurring one per meiosis, on one arm are matched by crossovers involving the same two strands on the other arm. Predictions from this model were that loci distant from the centromere would show positive interference, a high frequency of second-division segregations with some markers, and, for loci straddling the centromere, negative interference with an excess of two-strand double exchanges. For the ULG, the high frequency of Ts for crosses between *ac*17 and loci in the *fla*11 to *fla*13 region (Fig. 2) and the indications of positive interference in the same region (Table 2) are consistent with these requirements. For the centromere region the data from 2-factor crosses (Table 2) are of uncertain significance because of the small sample size. If the observed NPDs truly exceed expectation, they suggest either negative chiasma interfer-

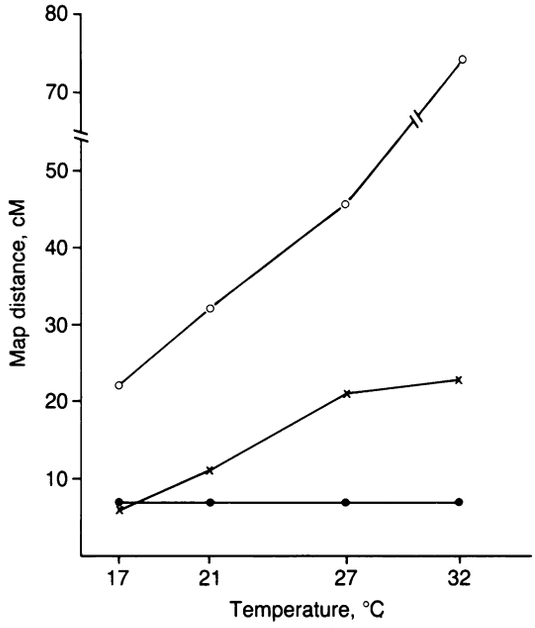

FIG. 3. Effect of phase II temperature on recombination. Crosses of *fla*9 *msr UNI*1, *SUN*1 *ERY*3 × *FLA*9 *MSR uni*1 *sun*1 *ery*3 were made carrying out phase II of the cycle at the temperatures shown. Map distance was analyzed from tetrad analysis (36 tetrads for each point). ○, *sun*1–*uni*1; ×, *uni*1–*fla*9; ●, *msr*–*ery*3.

Table 2. Comparison between observed NPD tetrads and those expected in the absence of genetic interference

Interval	Observed tetrad numbers			Expected NPD number
	PD	T	NPD	
*uni*1–*fla*9	235	91	1	4
*fla*10–*pf*10	43	59	0	8.3
*fla*10–*pf*29	14	21	6	2.3
*pf*10–*sun*1	55	44	5	3.4
*pf*29–*uni*1	14	20	4	2.3
*sun*1–*uni*1	41	56	0	7.8

The expected number of NPD tetrads was calculated as described in the text.

Table 3. Effect of phase II temperature on recombination

Interval	Linkage group	Map distance, cM			
		17°C	21°C	25°C	32°C
*fla*9–*fla*10	ULG	5	9	ND	26
*fla*10–*pf*10	ULG	15	18	23	36
*pf*10–*sun*1	ULG	16	34	ND	UL
*pf*16–*pf*13	IX	62	67	57	63
*pf*12–*pf*18	II	17	11	11	14

Each map distance was determined by analysis of 35–40 tetrads. ND, not determined; UL, unlinked.

Table 4. Recombination in *UNI FLA* × *uni1 fla9* zygotes in which parental strains are products of crosses at different phase II temperatures

F_1 zygote phase II temperature, °C	Temperature at which parentals were produced		
	17°C	21°C	32°C
17	30:0:4 6	25:0:5 8	11:0:2 8
21	22:0:9 15	17:0:7 15	12:0:5 14
32	17:1:17 35	15:0:15 25	9:0:8 24

Each entry gives tetrad numbers PD:NPD:T followed by map distance in cM.

ence or a possible excess of four-strand double exchanges. The occurrence of two-strand doubles cannot be determined from these 2-factor data.

It is apparent, however, that the restrictions on chromatid exchanges required for the linear chromosome model resemble those required to resolve potential dicentrics in the ring chromosome model discussed previously. In the linear model the number of exchanges per meiosis are limited and it is more difficult with this model than with the ring model to explain the extensive increases in ULG recombination when zygotes are exposed to higher temperatures. Complete understanding of the structure of the ULG will require isolation of its physical entity.

Stahl (17) has suggested that circular linkage may be selectively advantageous because it tends to preserve linkage between genes that may interact intimately in the same step in development. In the vegetative cycle of *C. reinhardtii* the postmitotic conversion of centrioles to basal bodies (18) may require this kind of coordinate activity. Whether it is a ring chromosome or a linear chromosome in which chromatid exchanges are constrained to give a circular map, the ULG with its cluster of genes that may effect specifically basal body assembly could represent this kind of specialized linkage function. However, only in the case of *uni* mutants is there good evidence that the gene product may function in the formation of the basal body transition zone (2).

For nearly a century, cell biologists have observed continuity between centrioles and basal bodies and, in many cases, reported apparent *de novo* origin of basal bodies (reviewed in refs. 19 and 20). It is possible that the ULG represents the genetic element frequently postulated to play a role in the cyclic formation of these structures.

Table 5. Recombination in *uni1 FLA9* × *UNI1 fla9* crosses in which temperature is varied early in phase II: Delay in shift to 32°C and limited shift to 32°C

Time, hr	Delay in shift to 32°C	Limited shift to 32°C
0.5	51:0:21 15	47:1:24 21
1	58:0:14 10	36:5:31 42
2	60:0:12 8	37:4:31 38
4	62:0:10 7	34:4:34 40
6	60:0:12 8	34:6:32 39
24	62:0:10 7	35:4:33 40
Control*	17°C 61:0:11 8	32°C 34:5:33 44

The data shown are taken from one of two experiments showing nearly identical results. In columns 2 and 3, zero time is the beginning of phase II. Each entry gives tetrad numbers PD:NPD:T followed by map distance in cM.

*In the control crosses, the entirety of phase II was carried out at the temperature indicated.

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