Short-Flagella Mutants of *Chlamydomonas reinhardtii*

Michael R. Kuchka[1] and Jonathan W. Jarvik

Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

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ABSTRACT

Six short-flagella mutants were isolated by screening clones of mutagenized Chlamydomonas for slow swimmers. The six mutants identify three unlinked Mendelian genes, with three mutations in gene *shf-1*, two in *shf-2* and one in *shf-3*. *shf-1* and *shf-2* have been mapped to chromosomes *VI* and *I*, respectively. Two of the *shf-1* mutations have temperature-sensitive flagellar-assembly phenotypes, and one *shf-2* mutant has a cold-sensitive phenotype. *shf shf* double mutants were constructed; depending on the alleles present they showed either flagellaless or short-flagella phenotypes. Phenotypic revertants of *shf-1* and *shf-2* mutants were isolated, and certain of them were found to carry extragenic suppressors, some dominant and some recessive. We suspect that the *shf* mutations affect components of a specific flagellar size-control system, the existence of which has been suggested by a variety of physiological experiments.

IT is a general property of flagellated or ciliated eucaryotic cells that they closely regulate ciliary or flagellar length. In the case of the biflagellate unicellular green alga *Chlamydomonas reinhardtii* the existence of a flagellar size-control system is implicit in a number of well-documented observations. These include: (1) Cells which have lost one or both of their flagella by amputation rapidly regenerate them to original length (ROSENBAUM and CHILD 1967; ROSENBAUM, MOULDER and RINGO 1969). (2) Cells whose flagella have shortened by resorption rapidly regenerate them to original length when resorption stimuli are removed (LEFEBVRE *et al.* 1978). (3) Certain quadriflagellate cells with unequal length flagella, which can be generated by mating long-flagella or short-flagella mutants to wild type, rapidly adjust all four flagella to wild-type length (JARVIK, LEFEBVRE and ROSENBAUM 1976; JARVIK *et al.* 1984). Taken together, these observations strongly suggest that the Chlamydomonas cell continuously monitors the lengths of its flagella and makes appropriate adjustments when necessary. Although the molecular nature of the flagellar size control system is unknown, we have previously obtained evidence that: (1) the size of the intracellular pool of flagellar protein does not limit the extent of flagellar growth (KUCHKA and JARVIK, 1982), and (2) an intrinsic inverse relation between flagellar length and the rate of flagellar growth does not limit the extent of flagellar growth (JARVIK *et al.* 1984).

To approach the molecular mechanism by which flagellar size-control is achieved, we have begun a genetic analysis of the phenomenon. In this communication we describe the identification of three genes—*shf-1*, *shf-2* and *shf-3*—which are required for normal flagellar size control in *C. reinhardtii*. Experiments which used *shf-1* mutants to test a model of size control were reported previously (JARVIK *et al.* 1984), and some of the experiments described here have been briefly summarized elsewhere (BALDWIN *et al.* 1985; JARVIK and KUCHKA, 1985).

MATERIALS AND METHODS

Strains and culture conditions: Wild-type strains used were the 137c derivatives *NO*, mt^+ and *NO*, mt^- provided by Dr. U. GOODENOUGH. Strains CC-1144 (*sr-1, msr-1, nr-1, act-2, pyr-1*, mt^-) and CC-638 (*ac-14, can-1, msr-1, act-2, sr-1, nic-76*, mt^+) (HARRIS, 1984) were provided by the Chlamydomonas Genetics Center, Duke University, Durham, NC. Cells were grown at 25° in Medium I of SAGER and GRANICK (1953) with constant aeration by bubbling. Cultures were illuminated with white light (45 cm from two General Electric F48T12-CW-HO fluorescent tubes) on a 14-hr light/10-hr dark cycle. Experiments were typically performed between hr 4 and 10 of the light segment of the cycle.

Mutagenesis and mutant isolation: Log phase cells were spread on 1.5% agar plates at a density of approximately 2000 cells/plate and shortly thereafter illuminated with ultraviolet light (60–120 sec at 48 cm from a General Electric G30T8 germicidal lamp). The plates were stored in the dark for 4 hr and then incubated at 25° in the light for 10 days. Survival was typically 10–20%. Individual colonies were picked from the plates with capillary tubes containing a small volume of liquid medium (JARVIK *et al.*, 1984) and each tube was observed at ×80 magnification with a dissecting microscope. Clones which showed slow motility were retained. All mutants were isolated in an *NO*, mt^+ (wild type) background except for *shf-2-158* which was isolated in a *cw-15* (cell wall-less) background (DAVIES and PLASKITT 1971).

Genetic analysis: Standard methods were used for preparing gametes and performing crosses (LEVINE and EBER-

[1] Present address: Department of Molecular Biology, University of Geneva, 30 quai E. Ansermet, CH 1211 Geneva 4, Switzerland.

SOLD, 1960). Marker-to-marker linkage was calculated using the relation (NPD + 1/2 T)/(PD + NPD + T). In some cases linkage was examined by scoring zygote colonies; here undissected tetrads were allowed to grow into macroscopic colonies which were picked into liquid media and scored for the presence of significant numbers (20% or more) of wild-type cells. Dominance tests were performed by measuring flagellar lengths in quadriflagellate cells fixed 2 hr after mixing mutant and wild-type gametes.

Isolation of revertants: The isolation of phenotypic revertants of *shf-1-253* was described previously (JARVIK *et al.* 1984). Selection for motile revertants of the flagellaless double-mutant *shf-1-253 shf-2-1249* was performed by spreading approximately 100 cells onto 1.5% agar plates and incubating them in the light at 25° until individual colonies were approximately 2 mm in diameter. The plates were irradiated with UV light for 60 sec and stored in the dark for 4 hr. Fifty colonies were then picked and transferred to 2-ml volumes of medium in 13 × 100 mm tubes. The tubes were incubated at 25° with illumination from above for 14 days. Cultures with cells at the meniscus were then streaked onto 1.5% agar plates and individual colonies were picked and reexamined microscopically to ascertain their motility phenotypes. No more than one motile isolate was retained from each tube to assure that all revertants were of independent origin.

Flagellar length measurements: Cells were fixed in 0.5% glutaraldehyde and flagellar lengths determined by phase contrast microscopy at ×800 magnification using an ocular micrometer. The flagella of at least 20 cells were measured for each sample, and mean flagellar lengths and standard deviations were computed.

Deflagellation regeneration and resorption: Deflagellation was achieved by shearing 10-ml cultures in a Waring blender for 15 sec. Flagellar regeneration kinetics were determined by fixing samples at intervals thereafter and measuring flagellar lengths as described above. Pool sizes were determined by deflagellating cells, incubating for two hours in the presence of 15 μg/ml cycloheximide, and measuring flagellar lengths. Flagellar resorption experiments were performed by adding 25 mM sodium citrate to log phase cultures and measuring flagellar lengths at intervals thereafter (LEFEBVRE *et al.* 1978).

RESULTS

Isolation and initial characterization of short-flagella mutants: Approximately 6000 UV-mutagenized clones were examined as described in MATERIALS AND METHODS. Twenty-six with slow swimming phenotypes were identified, and six of these proved to have short (approximately half-length) flagella. Flagellar length distributions in cultures of the six short-flagella (*shf*) mutants are shown in Figure 1; note that the mutant and wild-type length distributions showed very little overlap. The lengths of the two flagella on each cell were equal, and the mutants showed no obvious defects in flagellar waveforms when observed live or after glutaraldehyde fixation.

To look for temperature-sensitive or cold-sensitive phenotypes, cultures of each mutant were shifted from 25° to 13° or 34° and observed 48 hr later. Results are summarized in Table 1. Two mutants, *shf-1-236* and *shf-1-253*, proved to be temperature-sensitive for flagellar assembly, and one, *shf-2-158*, proved to be cold sensitive. Upon shift to nonpermissive temperature the conditional short flagella mutants did not immediately lose their flagella. Rather, most cells remained fully flagellated until, like wild type, they resorbed their flagella prior to mitosis and cell division. Unlike wild type, however, they typically failed to assemble new flagella after division. We previously reported that *shf-1-253* and *shf-1-277* are flagellaless when grown in medium containing millimolar sodium acetate and that flagellar resorption commences im-

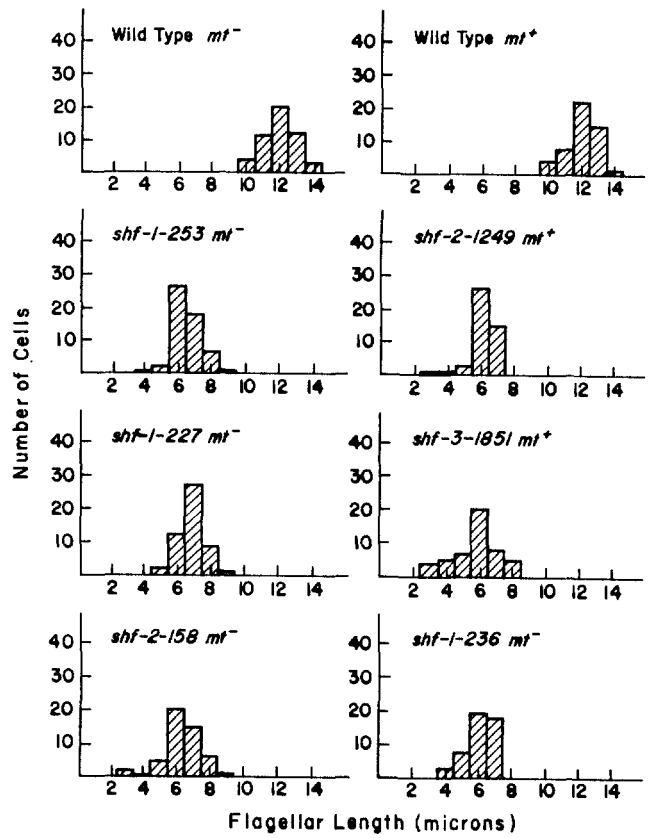

FIGURE 1.—Flagellar length distributions for *shf* mutants and for wild type.

TABLE 1

Conditional phenotypes of *shf* mutants

Mutant	Growth condition				
	13°	25°	34°	22 mM Acetate	2.2 mM Acetate
shf-1-236	Shf	Shf	Fla⁻	Fla⁻	Shf
shf-1-253	Shf	Shf	Fla⁻	Fla⁻	Fla⁻
shf-1-277	Shf	Shf	Shf	Fla⁻	Shf
shf-2-158	Fla⁻	Shf	Shf	Shf	Shf
shf-2-1249	Shf	Shf	Shf	Fla⁻	Shf
shf-3-1851	Shf	Shf	Shf	Shf	Shf

Temperature phenotypes were determined by diluting log phase cells growing at 25° into media at the temperatures shown and scoring after 2 days of growth. Acetate phenotypes were scored by diluting log phase cells into media supplemented with sodium acetate at the concentrations shown and scoring after 2 days of growth at 25°. Fla⁻ = flagellaless; Shf = short flagella.

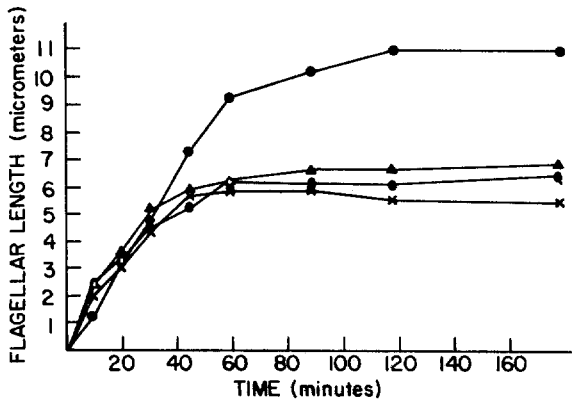

FIGURE 2.—Regeneration kinetics for wild type and *shf* mutants. *shf* mutations used were *shf-1-253*, *shf-2-1249* and *shf-3-1851*.

TABLE 2

Flagellar lengths and flagellar precursor pool sizes in vegetative and gametic *shf* mutants

	Flagellar length (μm)		Pool size (μm)	
Mutant	Vegetative	Gametic	Vegetative	Gametic
Wild type	12.8 ± 1.4	13.8 ± 1.4	6.4 ± 1.4	3.5 ± 1.3
shf-1-236	5.3 ± 0.8	5.0 ± 0.9	1.2 ± 0.9	1.4 ± 1.4
shf-1-253	6.6 ± 1.0	6.8 ± 1.1	1.0 ± 0.8	1.4 ± 1.0
shf-1-277	6.5 ± 0.8	6.6 ± 0.6	0.6 ± 0.9	1.1 ± 1.1
shf-2-1249	8.0 ± 1.4	6.8 ± 1.8	1.0 ± 1.1	2.2 ± 1.2
shf-3-1851	7.7 ± 1.4	7.6 ± 2.0	1.3 ± 1.2	1.9 ± 1.2

mediately upon the addition of acetate to the medium (JARVIK *et al.* 1984). Examination of the effect of sodium acetate on the flagellation phenotype of the rest of the *shf* strains indicated that all three *shf-1* mutants, and one of the two *shf-2* mutants, were more sensitive to acetate than wild type, with the threshold concentration varying from strain to strain (Table 1). The cellular or molecular basis for the acetate effect is unknown.

Kinetics of flagellar regeneration and resorption for *shf* mutants: All *shf* mutants were able to regenerate their flagella after deflagellation, and in each case flagellar regeneration followed kinetics which closely resembled those shown by wild type, except, of course, that the lengths plateaued at shorter values (Fig. 2). Like wild type, mutant cells contained pools of unassembled flagellar protein. Pool size was determined by measuring the extent of regeneration in the presence of the protein-synthesis inhibitor cycloheximide. As expected (Table 2), wild-type vegetative cells regenerated their flagella about half way when protein synthesis was inhibited, and gametes regenerated about one quarter of the way (ROSENBAUM, MOULDER and RINGO 1969, KUCHKA and JARVIK 1982). Pools in the *shf* mutants were smaller than wild type pools on a flagellar length basis, and, in contrast to wild type, *shf* vegetative cells appeared to have smaller pools than gametes (Table 2). The *shf* mutants were also tested for their resorption properties after chelation of free calcium with sodium citrate. Each resorbed with kinetics which closely resembled wild type's (data not shown).

TABLE 3

Complementation results for the *shf* mutations

	Complementation group					
	shf-1			shf-2		shf-3
Mutant	236	253	277	1249	158	1851
Wild type	+	+	+	+	+	+
236	−	−	−	+	+	+
253		−	−	+	+	+
277			−	+	+	+
1249				−	−	+
158					−	+
1851						−

Dominance and complementation tests: When *C. reinhardtii* mate, gametic cells of opposite mating type fuse in pairs to form temporary quadriflagellate dikaryons. If one of the parents is a flagellar mutant, observation of flagellar behavior in such quadriflagellates allows one to make an assessment of dominance or recessivity of the mutant phenotype (LEWIN 1954; STARLING and RANDALL 1971). Observation of such dikaryons for all *shf/wild type* combinations revealed that by 45 min post-mating, the two short flagella contributed by the *shf* parent had elongated to wild-type length. Because wild type size-control is imposed upon the two short flagella in these dikaryons, we conclude that each *shf* mutation is recessive in expression to wild type.

Complementation among the *shf* mutations was assessed by constructing and observing all possible *shf/shf* dikaryons. In such dikaryons, complementation is indicated by the outgrowth of all four flagella to wild-type length, and lack of complementation is indicated by the absence of such outgrowth. Based on such analyses, the six *shf* mutants fell into three complementation groups: *shf-1* (three alleles—236, 253, 277), *shf-2* (two alleles—158 and 1249), and *shf-3* (one allele—1851). Table 3 summarizes these results.

Tetrad analysis: When the *shf* mutants were crossed to wild type, 2:2 segregation of wild type and short progeny was consistently observed, indicating the presence of a Mendelian determinant for shortness in each mutant. Complete tetrads were examined for 42 *shf-1-236* × *wild type* cases, 48 *shf-1-253* × *wild type* cases, 13 *shf-1-277* × *wild type* cases, 51 *shf-2-1249* × *wild type* cases, and 21 *shf-3-1851* × *wild type* cases. When appropriate, additional phenotypic analyses of at least ten tetrads were done to confirm that the determinants of the other mutant phenotypes (temperature-conditionality and/or acetate-sensitivity) cosegregated with the *shf* mutation. The only mutant not analysed in parallel with the others here was *shf-*

TABLE 4

Linkage analysis for *shf* mutations

Cross	Markers scored	Ascus type PD	NPD	T
shf-1 × *shf-2*	*shf, shf*	3	7	14
shf-1 × *shf-3*	*shf, shf*	2	9	16
shf-2 × *shf-3*	*shf, shf*	14	11	15
shf-2 × CC 1144	*shf, msr*	11	0	5
shf-2 × CC 638	*shf, msr*	18	0	7

The *shf* mutations used in these crosses were *shf-1-253*, *shf-2-1249* and *shf-3-1851*. For cases where the *shf shf* phenotype proved to be short flagella (see Table 5) tetrads with 4 short: 0 full-length were scored as PDs; those showing 3:1 segregation were scored as Ts and those showing 2:2 segregation were scored as NPDs. For cases where the *shf shf* phenotype proved to be flagellaless, tetrads with 4 short: 0 full-length were scored as PDs; those with 2 short: 1 flagellaless: 1 full-length were scored as Ts; and those with 2 flagellaless: 2 full-length were scored as NPDs.

TABLE 5

***shf shf* double-mutant phenotypes**

Double mutant	Phenotype
shf-1-236 shf-2-1249	Fla$^-$
shf-1-236 shf-3-1851	Fla$^-$
shf-1-253 shf-2-1249	Fla$^-$
shf-1-253 shf-3-1851	Fla$^-$
shf-1-277 shf-2-1249	Shf
shf-1-277 shf-3-1851	Shf
shf-2-1249 shf-3-1851	Shf

2-158; this was because a number of nonflagellated *cw15*$^+$ segregants were observed in the *mutant/wild type* tetrads, suggesting the presence of a marker which produces a flagellaless phenotype in cells which have walls but not in those which do not. (Remember, *shf-2-158* is the only mutant which was isolated in a cell wall-less (*cw15*) background.)

Tetrad analysis of a number of *shf* × *shf* crosses indicated that the three *shf* genes are unlinked (Table 4). The tetrad data also allowed us to determine the phenotypes of a variety of *shf shf* double mutants. Depending on the alleles present, the *shf shf* double mutants had either short-flagella or flagellaless phenotypes at 25° (Table 5). For the three double mutants which had short flagella, backcrosses to wild type confirmed that each carried two *shf* mutations. For the four double mutants which were flagellaless, such confirmatory crosses could not be performed, since flagella are needed for mating in Chlamydomonas.

Mapping: We previously published data indicating that *shf-1* is on Chromosome *VI*, about 5 cM from the centromere (JARVIK *et al.* 1984). *shf-2* was mapped by crossing *shf-2-1249 mt*$^+$ to two multiply marked mapping strains, CC-1144 and CC-638. Tetrad data, shown in Table 4, indicate that *shf-2* is about 15 cM from *msr-1* on the right arm of Chromosome *I*. Analysis of a number of *shf-3/CC-1144* tetrads revealed no evidence of linkage of *shf-3* to *msr-1* (chromosome *I*), *act-2* (chromosome *VI*), *nr-1* (chromosome *VIII*), or *sr-1* (chromosome *IX*); *shf-3* thus remains unmapped at present. The limited tetrad data also indicate that *shf-3* is not closely linked to its centromere (*e.g.*, Table 4, line 2).

TABLE 6

Some *shf-1* revertants carry extragenic suppressors

Cross	Ascus type PD	NPD	T
Revertant 11 × wild type	3	10	2
Revertant 23 × wild type	9	0	0
Revertant 32 × wild type	15	0	0
Revertant 37 × wild type	5	6	0

Revertants 11 and 37 were revertant with respect to temperature sensitivity and flagellar length—*i.e.*, they had full-length flagella at all temperatures. Tetrads from crosses of these revertants to wild type were of three types. Those with four full length members were scored as PDs; those with two short and two full length members were scored as NPDs; those with one short and three full length members were scored as Ts. Revertants 23 and 32 were revertant only with respect to the temperature sensitive aspect of the parental phenotype—*i.e.*, they had short flagella at 13°, 25° and 34°. All tetrads from crosses of these revertants to wild type had two members with short flagella at all temperatures and two with wild-type flagella.

Isolation of revertants of *shf-1* that carry extragenic suppressors: *shf-1-253* is flagellaless at 34°. We previously described the isolation, by selection for motile phototactic cells at 34°, of 45 independent UV-induced phenotypic revertants of *shf-1-253* (JARVIK *et al.* 1984). In the present study, four revertants—numbers 11, 23, 32 and 37—were analyzed genetically to determine whether or not they might carry extragenic suppressor mutations along with *shf-1-253*. We obtained evidence, described below, that two of the strains do carry extragenic suppressors.

Each of the four revertants was crossed to wild type and the meiotic products were scored for flagellar length and for temperature-sensitive flagellar assembly. The results, shown in Table 6, indicate that revertants 11 and 37 carry suppressor mutations unlinked to *shf-1*. Segregants carrying the suppressor mutations alone had wild type length flagella and were not mutant with respect to temperature and acetate. This conclusion was confirmed by further crosses using the *shf*$^+$ *sup* segregants—see, for example, Table 8. Revertants 11 and 37 were backcrossed to *shf-1-253* to examine further the segregation of the suppressor mutations. All 24 revertant 11 × *shf-1-253* tetrads, and all 12 revertant 11 × *shf-1-253* tetrads segregated two short: two full length, indicating that suppression of *shf-1* is due to a single Mendelian mutation in each case. We have not done the appropriate crosses to determine whether the two suppressors are linked, nor have we attempted to map them.

Having established that revertants 11 and 37 carry

TABLE 7

Dominance tests for suppressor function

Genotype	Flagellar length (μm)
Wild type	12.5
shf-1-253	7.2
Shf-1-253 sup-r11	11.0
Shf-1-253 SUP-r37	10.8
Shf-1-253 sup-r11/wild type	10.4
Shf-1-253 sup-r11/shf-1-253	7.4
Shf-1-253 SUP-r37/wild type	11.6
Shf-1-253 SUP-r37/shf-1-253	11.5

TABLE 8

Function of suppressors of *shf-1-253* in other *shf-1* backgrounds

Cross	Ascus type		
	PD	NPD	T
shf-1-277 × revertant 37	18	0	0
shf-1-277 × revertant 11	5	3	6
shf-1-236 × *SUP-r37*	8	4	4
shf-1-236 × *sup-r11*	4	4	1

In the *shf-1-277* crosses, PDs segregate 2 short:2 wild type; NPDs segregate 4:0; Ts segregate 3:1. In the *shf-1-236* crosses, PDs segregate 2:2, NPDs segregate 0:4; Ts segregate 1:3.

extragenic suppressors, we proceeded to perform dominance tests for suppressor function in mutant/revertant quadriflagellate dikaryons (Table 7). If a suppressor is dominant to wild type, the shorter pair of flagella donated to the dikaryon by the *shf* parent should elongate; if it is recessive, the longer pair of flagella donated by the revertant should shorten. We observed that in revertant *37/shf-1-253* dikaryons, the shorter pair of flagella elongated from 7.2 to 12.5 μm. The suppressor mutation in revertant 37 is therefore dominant to its wild-type allele; we denote the mutation *SUP-r37*. Conversely, in revertant 11/*shf-1-253* dikaryons, the longer pair of flagella shortened from 11.0 μm to 7.4 μm. The suppressor mutation in Revertant 11 is therefore recessive; we denote it *sup-r11*.

The behavior of the *SUP-r37* and *sup-r11* mutations in other *shf-1* mutant backgrounds was investigated. *shf-1-277* was crossed to revertants 11 and 37 and tetrads were scored for flagellar length. *shf-1-277* × revertant 37 yielded 18 tetrads, all of which segregated 2 short: 2 wild type. Since *SUP-r37* is unlinked to *shf-1*, we conclude that it is equally capable of suppressing *shf-1-253* and *shf-1-277*. The cross *shf-1-277* × revertant 11 generated tetrads which segregated short-to-wild type in 2:2, 4:0, or 3:1 ratios. This indicates that *sup-r11* does not suppress the *shf-1-277* allele. Strains bearing suppressors *SUP-r37* and *sup-r11* alone were crossed to *shf-1-236* and tetrads were analyzed. Segregation patterns indicated suppression of the *shf-1-236* short flagella phenotype by both *SUP-r37* and *sup-r11*. To summarize, the dominant *SUP-r37* mutation suppresses all three *shf-1* alleles. The recessive *sup-r11* mutation suppresses *shf-1-236* and *shf-1-253* but is not able to suppress *shf-1-277*. Tetrad data indicating the effects of *SUP-r37* and *sup-r11* in combination with *shf-1-236* and *shf-1-277* are presented in Table 8.

Revertants of *shf-2*: Since the *shf-1-253 shf-2-1249* and *shf-1-253 shf-3-1851* double mutants were flagellaless (Table 5), strong selective pressure could be applied to these strains to obtain phenotypic revertants which are motile and phototactic. In the *shf-1-253 shf-2-1249* background, 24 UV-induced independent phenotypic revertants were isolated as described in MATERIALS AND METHODS. Which *shf* mutation was involved in a given reversion event can be determined by backcrossing the revertant to one or the other *shf* parent and screening progeny for wild type segregants. Crossing a revertant by *shf-2-1249* will yield wild-type progeny only if reversion is by back mutation (or pseudoreversion) at *shf-2-1249* or by suppression of *shf-2-1249*. Likewise, wild type recombinants can be generated in crosses to *shf-1-253* only if reversion is by back mutation at *shf-1-253* or suppression of *shf-1-253*.

Two representative revertants generated in the *shf-1-253, shf-2-1249* background were backcrossed to *shf-2-1249*. These revertants, R19 and R20, had short flagella, and so both participants in each cross had short flagella. Colonies derived from individual zygotes were picked into liquid medium and the cells scored for flagellar length. Of 30 zygote colonies examined from the R19 cross, 27 contained a substantial proportion of cells with full length flagella. Evidence that the cells really came from zygotes was obtained by washing them into medium without nitrogen and observing that many cells were mating 14 hr later, just as ought to happen in a mixed culture containing cells of both mating types. One hundred zygote colonies were analyzed from the R20 cross. Fifteen showed significant numbers of cells with full length flagella. Again, all of these cultures showed self-mating after starvation for nitrogen, indicating that they were of meiotic origin. These results indicate that in both R19 and R20, reversion or suppression of the *shf-2* mutation, and not the *shf-1* mutation, has occurred.

DISCUSSION

Our results suggest that, rather than having lost the ability to regulate flagellar length, the *shf* mutants regulate length much like wild type but to abnormally short values. For each mutant, the distribution of flagellar lengths around the mean was similar to that of wild type, though, of course, the mean was smaller. The mutants regenerated and resorbed their flagella

with normal kinetics and contained substantial pools of unassembled flagellar protein (Figure 2, Table 2). Although mutant cells swam more slowly than wild type due to their shorter flagella, their motility functions were apparently normal: mutant cells were phototactic, they were capable of both forward and backward swimming, and they were proficient in mating, a process which involves specific flagellar interactions in Chlamydomonas.

We identified multiple alleles of two different *shf* genes and a single allele of a third. This may mean that the system of size control in the Chlamydomonas flagellum is fairly simple; that is, regulation of flagellar size may involve a relatively small number of gene products. However, we by no means can claim to have reached saturation for *shf* genes, and isolation and analysis of additional *shf* mutants is clearly called for. Furthermore, the short-flagella phenotype may serve to identify only a subset of the genes involved in flagellar size control, and so isolation and analysis of long-flagella mutants (McVittie 1972; Jarvik, Lefebvre and Rosenbaum 1976), as well as further analysis of suppressors of *shf* mutations, is also in order.

Wild-type vegetative and gametic cells contain pools of unassembled flagellar protein (Rosenbaum, Moulder and Ringo 1969; Lefebvre *et al.* 1978). We found (Figure 2) that all *shf* mutants maintained pools of unassembled flagellar protein. Although these pools were smaller than in wild type, extra flagellar protein was available for assembly but not used, and so we can rule out the possibility that *shf* flagella are short simply because mutant cells make too little flagellar protein. This conclusion is supported by the behavior of *shf-1-253 sup-r11/wild type* quadriflagellates (Table 7). Here the two longer flagella shortened to *shf* length, even though, by so doing, they augmented the intracellular flagellar protein pool.

We constructed a variety of *shf shf* double mutants in order to study the interaction of the various mutations. Depending on the alleles present, double mutant cells either carried short flagella or were flagellaless. It is difficult to interpret these results in mechanistic terms, as no specific molecular components of the size control system have yet been identified. However, we feel safe in stating that the maintenance of proper flagellar length involves the products of a number of different genes and that these gene products must interact in some direct or indirect way to regulate flagellar length. Since flagellar length control must be intimately associated with flagellar assembly, and because components of a length-determining system might themselves be axonemal proteins or factors involved in flagellar assembly, it is not surprising that certain double mutant combinations are assembly-defective. *shf* mutations which, when paired, confer a short-flagella phenotype to the double mutant may represent obstructions in different steps of the same pathway of gene product interaction. For example, both the *shf-2* and the *shf-3* mutations confer a short-flagella phenotype to the mutant cell. The *shf-2 shf-3* double mutant also has short flagella, so this combination of mutations has no more apparent effect on the cell than does each individual mutation. This might mean that *shf-2* and *shf-3* mutations block steps in the same pathway of protein interaction involved in flagellar length regulation. Conversely, certain alleles of *shf-1*, when paired with either *shf-2* or *shf-3*, accord a flagellaless phenotype to the double mutant. The *shf-1* mutation might block a step in the pathway distinct from the one which involves the *shf-2* gene product. With both pathways blocked, a cumulative effect would be seen in the double mutant—*e.g.*, the flagellaless state of the *shf-1-253 shf-2-1249* double mutant cell. This interpretation, though attractive, does not easily accomodate the fact that the phenotype is allele specific with respect to *shf-1* (*e.g.*, *shf-1-253 shf-2-1249* is flagellaless and *shf-1-277 shf-2-1249* is not). More about the biochemistry of size control must be known before these results can be understood in meaningful detail.

Extragenic suppressor mutations have been identified in two revertants of *shf-1-253*. These suppressors behave quite differently. *SUP-r37* is dominant to its wild-type allele in quadriflagellate dikaryons and is capable of suppressing all three alleles of *shf-1*. *sup-r11* is recessive and is able to suppress *shf-1-236* and *shf-1-253* but not *shf-1-277*. Our purpose in isolating and analyzing phenotypic revertants of *shf* mutants was to identify, by way of extragenic suppression, genes whose products might functionally interact with the products of the known *shf* genes (Jarvik and Botstein, 1975; Moir *et al.* 1982), and/or to identify, by way of intragenic pseudoreversion, additional mutations in the known *shf* genes (Luck *et al.* 1977). In this context we view the results described here as serving primarily to demonstrate that our methods are effective in selecting revertants, some with extragenic suppressors. Additional work would clearly be required before we could say that the suppressors are in new genes with specific size-control functions. Rather than examine these revertants further, however, we feel that it would be more profitable to isolate and analyze revertants with distinctive flagellar phenotypes such as long flagella, abnormal flagellar motility or temperature-sensitive flagellar assembly, since genetic analysis is simplified whenever the mutations under study have distinctive phenotypes.

The problem of size control at the cell or large-organelle level has received some experimental attention to date, but the molecular mechanisms of size

control for large organelles or for cells themselves remain largely enigmatic. Among the investigations of size control presently underway, the most advanced is probably the analysis of cellular size control in the fission yeast *Schizosaccaromyces pombe* (NURSE 1985). We find it curious, and possibly significant, that, as with short-flagella genes in Chlamydomonas, there appear to be just a few genes in *S. pombe* in which small-cell mutations can easily be isolated. Perhaps the size-control mechanisms for the flagellum and for the cell itself are similar, or even homologous.

We have shown in this communication that we can isolate short-flagella mutants in Chlamydomonas and analyze them by standard genetic means. The results presented here are purely genetical, and as such they do not provide evidence about the specific molecular mechanism of flagellar size control. However, it should be clear to the reader that the examination of the ultrastructure and protein-composition of the mutants might serve to implicate particular structures, or particular proteins, in the size-control process.

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