

# Analysis of Flagellar Size Control Using a Mutant of *Chlamydomonas reinhardtii* with a Variable Number of Flagella

MICHAEL R. KUCHKA and JONATHAN W. JARVIK

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

**ABSTRACT** A mutant of *Chlamydomonas reinhardtii* with a variable number of flagella per cell has been used to investigate flagellar size control. The mutant and wild-type do not differ in cell size nor in flagellar length, yet the size of the intracellular pool of flagellar precursor protein can differ dramatically among individual mutant cells, with, for example, triflagellate cells having three times the pool of monoflagellate cells. Because cells of the same size, but with very different pool sizes, have flagella of identical length, it appears that the concentration of the unassembled flagellar precursor protein pool does not regulate flagellar length. The relation between cell size, pool size, and flagellar length has also been investigated for wild-type cells of different sizes and ploidies. Again, flagellar length appears to be maintained independent of pool size or concentration.

Size control is an essential concern for any cell, and yet the way in which a cell controls size is not understood in even a single case. The importance of a size regulation system is obvious for growing cells, because growth and division must be precisely coordinated to maintain cell size from generation to generation (3, 4, 26). Whatever the molecular mechanism of size control may be, a number of investigations clearly show that its determination is a homeostatic phenomenon in which cell size anomalies, whether induced naturally (9) or experimentally (20), are corrected.

Like total cell size, organelle size must also be controlled to maintain balanced cell growth, or to express particular differentiated states. The flagella of the biflagellate unicellular eucaryote *Chlamydomonas reinhardtii* are organelles whose size and number are closely regulated. Amputation of one or both flagella triggers a host of complex events, including commencement of flagellar assembly through the mobilization of an intracellular flagellar precursor protein pool (21), transcription of specific flagellar protein genes (12, 25), translation of the newly transcribed messages (12), use of the newly synthesized protein to continue flagellar regeneration (11, 21), cessation of net flagellar assembly at the appropriate length (11, 21), replenishment of the intracellular pool (11), and cessation of specific transcription/translation (11). As a consequence of these events, the original state of the cell is restored within 90 min of amputation. The complex response by *C. reinhardtii* to flagellar amputation may involve flagellar size detection both

at its beginning, when the detection of flagellar shortness may lead to the mobilization of the pool for assembly, and at its end, when the detection of flagellar full-length may lead to the cessation of net flagellar assembly.

Flagellar size control in *Chlamydomonas* is not confined to periods of flagellar growth or regeneration; rather, it appears to be a constant cellular activity. Thus, if the flagella are caused to resorb partially and then are released from resorption conditions, normal length is quickly restored (11). Or, when quadriflagellate dikaryons are constructed by mating wild-type with certain long (6) or short (7) flagella mutants, normal length control is rapidly imposed, with the long or short flagella being adjusted to wild-type length. Therefore, it appears that the cell constantly monitors the length of its flagella and takes appropriate action if the length is not right.

In this paper we describe experiments using a mutant with an abnormal flagellar number, and using cells that differ from one another in size but not in flagellar number, to test the hypothesis that the cell regulates flagellar length by controlling the level of some assembly-limiting component of the precursor protein pool. The results show that pool size and flagellar length are not dependent on one another in any simple way, thereby suggesting that the hypothesis is invalid. Other recent results from our laboratory, based on experiments using a short-flagella mutant, indicate the same thing, and further suggest that flagellar length is not dictated by some intrinsic property of flagellar assembly whereby longer flagella assemble

less readily (7). The basis for flagellar length control thus remains an intriguing mystery.

## 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 CC47 ( $arg-2\ mt^+$ ) and CC51 ( $arg-7\ mt^-$ ), which were used for diploid construction, and strain CC530 ( $ac-17\ mt^-$ ), which was used for centromere mapping, were provided by the Chlamydomonas Genetics Center, Duke University, Durham, N. C. Cells were grown at 25°C in medium I of Sager and Granick (22) bubbled continuously with air. Cultures were illuminated with white light (45 cm from two General Electric F48T12-CW-HO fluorescent tubes; General Electric Co., Wilmington, Mass.) on a 14-h light/10-h dark cycle. Experiments were typically performed between hours 4 and 9 of the light segment of the cycle.

### Mutagenesis and Mutant Isolation

Log phase NO  $mt^+$  cells were spread on 1.5% agar plates and illuminated with ultraviolet light (120 s at 48 cm from a General Electric Co. G30T8 germicidal lamp). Survival was ~6%. Plates were stored in the dark for 4 h and then incubated at 25°C for 10 d. Individual colonies were picked from the plates with capillary tubes containing liquid media, and each tube was observed at a magnification of 80 times in a dissecting microscope (8). Strains showing slow or aberrant motility were retained.

### Genetic Analysis

Standard methods were used for the preparation of gametes and for performing crosses (10, 13). Diploids were constructed by crossing haploid strains and selecting on minimal medium (2). Diploids were identified on the basis of three properties: prototrophy, large cell size, and minus mating type. Centromere linkage was calculated from crosses to an  $ac-17$ -strain using the formula ( $\frac{1}{2}$  T)/NPD + PD + T (16).

### Flagellar Length and Cell Size Determinations

Cells were fixed in 0.5% glutaraldehyde, and flagellar lengths were determined by phase-contrast microscopy at 800-times magnification using an ocular micrometer. The flagella of 20 cells were typically measured for each sample, and the average length was computed. Cell volumes were determined by making the simplifying assumption that cells are ellipsoidal in shape. The long and short dimensions of the cell were measured and the relation volume:  $\pi/6$  (short dimension)<sup>2</sup>(long dimension), was applied.

### Deflagellation and Flagellar Regeneration

Deflagellation was achieved by shearing 10 ml of culture in a Waring blender (Waring Products, New Hartford, Conn.) for 15 s. Cells were returned to normal culture conditions, and flagellar lengths on individual fixed cells were measured at intervals. For protein-synthesis inhibition experiments, cultures were divided immediately after deflagellation, and 15  $\mu$ g/ml cycloheximide was added.

### Mitotic Pedigree Analysis

A dilute synchronous cell culture was suspended in liquid 0.5% agar. A drop of this suspension was placed on a glass slide, covered with a glass cover slip, and the edges of the cover slip were sealed with Vaseline. Cell positions were recorded using the vernier scale on the microscope stage, and the flagellar number of each cell was also recorded. The slides were then maintained on the light/dark cycle overnight, during which individual cells underwent one, two, or, sometimes, three rounds of mitosis. The next morning, daughter or granddaughter cells were observed where individual cells had been before division, and the number of flagella on each was recorded.

## RESULTS

### Isolation and Initial Characterization of a Mutant with Variable Flagellar Number

Wild-type *C. reinhardtii* was mutagenized with ultraviolet light, and individual clones were screened for aberrant motility. Mutant 220 was first identified on the basis of a complex

motility phenotype; some cells were nonmotile whereas others showed aberrant swimming. On closer inspection, the mutant was found to have a variable number of flagella per cell, with the number of flagella on individual cells varying from zero to six. A frequency distribution for cells of different flagellar number is shown in Fig. 1. This distribution was found to be the same for vegetative cells or for gametes. A Nomarski differential interference contrast micrograph showing two mutant cells, one with two flagella and one with four flagella, is shown in Fig. 2; note that, as in wild-type cells, all the flagella of the mutant originate at the anterior end of the cell. Cell size in a growing population is variable but no more so than for wild-type, ranging from ~100  $\mu$ m<sup>3</sup> in small cells to ~600  $\mu$ m<sup>3</sup> in large cells. There is no apparent relationship, however, between cell size and flagellar number—i.e., a large cell is no more likely to have excess flagella than a small cell. It is important to point out that, although mutant 220 is abnormal with respect to flagellar number, it is quite normal with respect to flagellar length. The original  $vfl-2-220$  isolate was used in all experiments reported here, except where noted otherwise.

### Genetic Analysis

34 mutant  $\times$  wild-type tetrads were dissected, and in each the variable flagellar number trait segregated 2:2. Thus, the mutant phenotype appears to represent the expression of a single mendelian mutation. We have named the gene carrying the mutation  $vfl-2$  (for variable number of flagella), and we denote the mutation under study here as  $vfl-2-220$ . To determine centromere linkage, a  $vfl-2-220\ mt^+$  strain was crossed to a  $ac-17\ mt^-$  strain, and 25 tetrads were dissected and analyzed. The  $ac-17$  marker is tightly linked to its centromere on linkage group III. Of the 25 tetrads, 6 were parental ditype (PD), 8 were nonparental ditype (NPD), and 11 were tetratype (T).  $Vfl-2$  is thus unlinked to  $ac-17$ , and is ~22 map units from its own centromere. Based on this analysis,  $vfl-2$  appears to be distinct from  $vfl-1$ , which was isolated by M. Adams and D. Luck and which has been located 3 map units from the centromere on the right arm of linkage group VII (M. Adams, personal communication).

### Single Cell Pedigree Analysis

Single  $vfl-2-220$  cells were immobilized in 0.5% agar, and flagellar number was recorded before and after cell division.

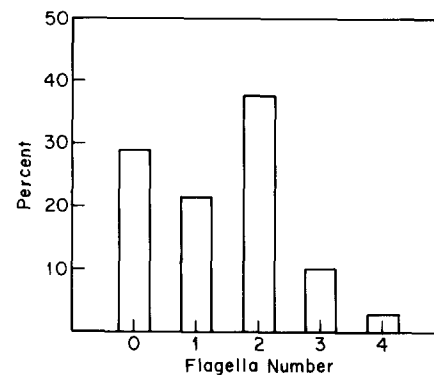


FIGURE 1 Frequency distribution of flagellar number on vegetative mutant cells. Cells with five or six flagella were present at a frequency of ~0.1%. A grand total of 2,162 cells were scored. In wild-type cultures, cells with two flagella typically make up >95% of the total and cells with 1 and 3 flagella always make up <1%.

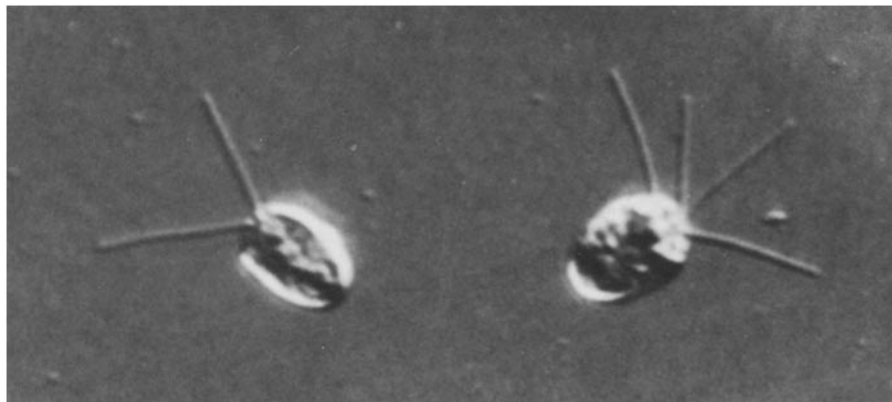


FIGURE 2 Nomarski differential interference contrast micrographs of two *vfl-2-220* cells.  $\times 2000$ .

The results for 15 randomly chosen cells are shown in Table I. There seems to be no general rule of segregation for flagellar number in the mutant cells. In just these 15 cases, cells with 2 flagella gave rise to daughters or granddaughters with 0, 1, 2, 3, or 4 flagella; cells with 1 flagellum produced cells with 0, 1, or 2 flagella; cells with 3 flagella gave progeny with 0 or 2 flagella; and cells with no flagella gave rise to progeny with 0, 1, or 2 flagella. These data demonstrate that it is possible to obtain the entire spectrum of flagellar number classes from a single *vfl-2-220* cell in just a few generations.

Two properties of mutant populations are consistent with the above observations. First, all clones derived from single mutant colonies show identical distributions of flagellar number classes; it is not possible to clone a line that is pure with respect to flagellar number. Second, when mutant cultures are grown continuously over periods of many weeks with repeated dilution to fresh medium, the distribution of flagellar number classes remains constant.

### Flagellar Regeneration in *vfl-2-220* and Wild-type Cells

Cultures of *vfl-2-220* and wild-type cells were deflagellated and the kinetics of regeneration, with and without new protein synthesis, were determined. Flagellar number, as well as flagellar length, was scored for each mutant cell examined. The results are plotted in Fig. 3. They can be easily summarized by stating that neither the kinetics of regeneration nor the final lengths achieved show any dependence on flagellar number. Furthermore, the distribution of flagellar number classes in the mutant cultures was the same before and after regeneration, suggesting that each cell regenerates the same number of flagella with which it began.

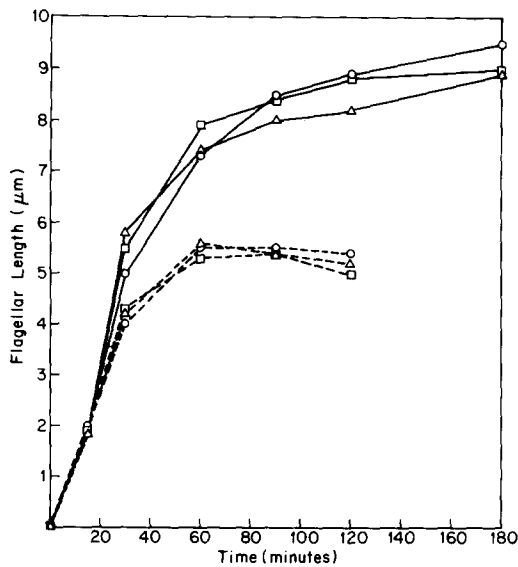
To examine whether or not the partial regeneration that takes place in the absence of protein synthesis is due to the exhaustion of some limiting pool component, two redeflagellation experiments were performed. In the first experiment, cells were deflagellated, allowed to regenerate for 120 min (i.e., to completion), deflagellated a second time, and, finally, allowed to regenerate for an additional 180 min. Regenerations were carried out in the presence and absence of cycloheximide. Cycloheximide, when present, was added at the time of the first deflagellation and was maintained continuously in the culture thereafter. It was found that the cells in cycloheximide failed to regenerate at all after the second deflagellation, suggesting that they had fully exhausted some component necessary for assembly. They had not lost the potential to regenerate, however, because if they were washed free of cycloheximide

TABLE I  
Single Cell Pedigree Analysis of Mutant *vfl-2-220* cells

Number of flagella on parent cell	Number of flagella on daughter cells
2	3, 3, 1, 2
0	0, 0, 0
1	2, 1
2	2, 0
0	2, 0
2	2, 2, 0
2	2, 2, 2, 1
1	2, 0, 0
2	1, 2
2	4, 0
3	2, 0
2	2, 2, 2, 2, 2, 2
3	2, 0
1	2, 1
0	2, 1, 1, 1

after the second deflagellation, they regenerated full length flagella.

A redeflagellation experiment similar to the one just described was also performed, but here the second deflagellation was scheduled after just 20 min of regeneration, at a time before the flagella had stopped growing. This time, flagellar regeneration occurred after both deflagellations in the presence of cycloheximide, and the length achieved after the second regeneration had reached completion was such that the total amount of flagellar growth from both regenerations was very nearly equal to the amount achieved when the first regeneration was allowed to go to completion (Table II). These results again suggest that the flagellar length achieved in cycloheximide is determined by some limiting component of the unassembled precursor protein pool, and they show that more than one initiation of flagellar assembly can take place in the absence of protein synthesis. The possibility that the above results represent a time-dependent decay, in the presence of cycloheximide, of cellular regeneration capacity—a decay that would occur with or without flagellar growth—was ruled out in the following experiment. NO *mt*<sup>+</sup> cells were preincubated for 1 h in cycloheximide medium, deflagellated, and monitored for flagellar regeneration in the same medium. Regeneration was observed, and its extent was comparable to that of nonpreincubated controls. (Predeflagellation lengths:  $11.0 \pm 1.5 \mu\text{m}$  with preincubation and  $11.4 \pm 1.2 \mu\text{m}$  without. Flagellar lengths 90 min after deflagellation:  $6.0 \pm 1.2 \mu\text{m}$  with preincubation and  $5.0 \pm 1.5 \mu\text{m}$  without.)



*vfl-2-220* Regeneration Kinetics

FIGURE 3 Flagellar regeneration kinetics of mutant *vfl-2-220* cells with 1, 2, or 3 flagella in the presence (dashed lines) and absence (solid lines) of cycloheximide. (○) cells with 1 flagellum; (□) cells with 2 flagella; (△) cells with 3 flagella. Each point represents measurements of 20 cells. Standard deviations of all points were <1.8  $\mu\text{m}$ .

TABLE II  
Flagellar Lengths Attained After One or Two Deflagellations in Cycloheximide

Cell Type	Original length $\mu\text{m}$	Length 20 min after deflagellation $\mu\text{m}$	Final length at 180 min	
			Deflagellation at 0 and again at 20 min $\mu\text{m}$	Deflagellation only at 0 $\mu\text{m}$
<b>With cycloheximide</b>				
Wild type	9.4 $\pm$ 1.2*	1.3 $\pm$ 1.0	1.6 $\pm$ 1.1	3.0 $\pm$ 1.3
<i>vfl-2-220</i> monoflagellates	9.7 $\pm$ 1.3	1.9 $\pm$ 0.8	2.4 $\pm$ 1.0	4.1 $\pm$ 1.2
<i>vfl-2-220</i> biflagellates	9.7 $\pm$ 1.3	1.6 $\pm$ 0.6	2.5 $\pm$ 0.6	3.7 $\pm$ 1.0
<i>vfl-2-220</i> triflagellates	9.4 $\pm$ 1.6	1.7 $\pm$ 0.8	2.1 $\pm$ 0.7	3.5 $\pm$ 1.1
<b>Without cycloheximide</b>				
Wild type	9.4 $\pm$ 1.2	2.2 $\pm$ 1.1	7.8 $\pm$ 1.2	8.5 $\pm$ 1.2
<i>vfl-2-220</i> monoflagellates	9.7 $\pm$ 1.3	2.4 $\pm$ 1.2	8.4 $\pm$ 1.3	8.9 $\pm$ 1.3
<i>vfl-2-220</i> biflagellates	9.7 $\pm$ 1.3	2.3 $\pm$ 0.5	8.3 $\pm$ 1.3	8.4 $\pm$ 1.0
<i>vfl-2-220</i> triflagellates	9.4 $\pm$ 1.6	2.0 $\pm$ 0.6	9.1 $\pm$ 1.5	8.6 $\pm$ 1.5

Drug was added at time of first deflagellation and was maintained in cultures thereafter. Each point represents measurements of 20 cells.  
\* Lengths expressed  $\pm$  SD.

### Measurements of Flagellar Length, Pool Size, and Cell Size in Diploid and Haploid Cells

The sizes of the flagellar precursor protein pools for diploid and haploid vegetative and gametic cells of different cell volumes were determined. Cell body size was scored along with flagellar length before and after deflagellation in the presence

TABLE III

Flagellar Lengths of Haploid and Diploid Vegetative and Gametic Cells Attained After Regeneration in the Presence and Absence of Cycloheximide

Cell type	Cell size $\mu\text{m}^3$	Percent of total	Flagellar Length		
			Predeflagellation $\mu\text{m}$	Postdeflagellation	
			without cycloheximide $\mu\text{m}$	with cycloheximide $\mu\text{m}$	
<b>Haploid vegetative</b>					
	0-50	0	—	—	—
	50-150	29	10.9 $\pm$ 0.8	8.6 $\pm$ 0.9	5.3 $\pm$ 0.9
	150-250	40	11.6 $\pm$ 1.1	9.3 $\pm$ 1.0	
	250-350	7	11.3 $\pm$ 1.4	10.8 $\pm$ 0.8	7.2*
	350-450	10	11.3 $\pm$ 0.9	9.6*	6.7 $\pm$ 1.8
	450-550	12	11.2 $\pm$ 0.9	10.6 $\pm$ 1.2	6.6*
	550-750	1	—	—	—
<b>Diploid vegetative</b>					
	0-50	0	—	—	—
	50-150	0	—	—	—
	150-250	16	10.8 $\pm$ 0.9	10.4 $\pm$ 1.1	6.3 $\pm$ 1.0
	250-350	12	12.0*	11.6 $\pm$ 0.6	5.9 $\pm$ 1.7
	350-450	40	11.8 $\pm$ 0.9	11.3 $\pm$ 1.0	6.9 $\pm$ 0.8
	450-550	19	12.1 $\pm$ 1.3	12.5 $\pm$ 1.0	7.2 $\pm$ 1.4
	550-750	13	13.2 $\pm$ 0.9	12.8 $\pm$ 1.1	7.4 $\pm$ 1.8
<b>Haploid gametic</b>					
	0-50	5	—	—	—
	50-150	41	12.8 $\pm$ 1.0	9.8 $\pm$ 1.0	3.5 $\pm$ 1.6
	150-250	41	13.9 $\pm$ 1.0	10.7 $\pm$ 1.0	5.0 $\pm$ 1.1
	250-350	9	13.6 $\pm$ 1.5	11.7 $\pm$ 0.5	5.7*
	350-450	4	—	—	—
	450-550	0	—	—	—
	550-750	0	—	—	—
<b>Diploid gametic</b>					
	0-50	0	—	—	—
	50-150	26	11.4 $\pm$ 1.0	9.2 $\pm$ 1.0	5.3 $\pm$ 1.7
	150-250	45	12.4 $\pm$ 0.8	10.5 $\pm$ 1.1	6.5 $\pm$ 1.1
	250-350	7	12.0*	11.4 $\pm$ 1.1	6.6 $\pm$ 1.8
	350-450	13	13.1 $\pm$ 0.9	11.0 $\pm$ 0.9	8.1 $\pm$ 1.5
	450-550	9	13.6 $\pm$ 0.6	11.7 $\pm$ 1.2	8.2 $\pm$ 1.7
	550-750	0	—	—	—

Measurements were made on 50 cells in each sample so that cell-size distribution data were derived from 150 cells total.

\* Standard deviations are not given for sample sizes smaller than five.

and absence of cycloheximide. Table III shows average length values for cells of different sizes, and Fig. 4 shows representative data from the same experiments displayed in scatter plot form. Although there is a rather broad distribution of cell size in both haploid and diploid cells, diploid vegetative and gametic cells are, on average, larger than corresponding haploid cells (2). Within a specific cell volume class, there is little or no difference between the flagellar lengths of haploid and diploid gametes, and the same can be said for haploid and diploid vegetative cells. Nor is there a consistent difference in pool size between haploid and diploid vegetative cells of the same size. There may be some positive correlation between cell size and pool size, particularly for gametes, but the scatter in the data is such that this relationship cannot be used to predict the pool size of any particular cell (Fig. 4).

### DISCUSSION

#### Flagellar Length Is Not Dependent on Pool Size

The eucaryotic axoneme is extremely complex, containing well in excess of 100 structural proteins (19). However, because

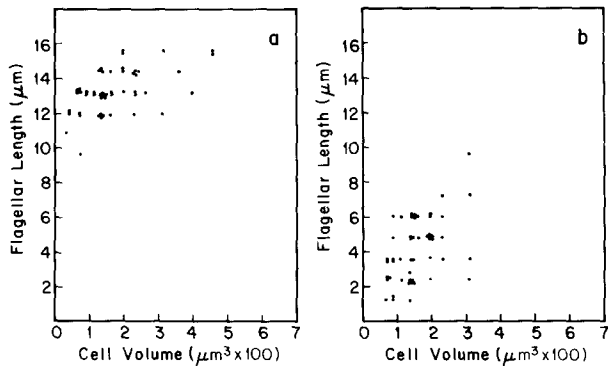


FIGURE 4 Relationship between cell volume and flagellar length for 50 *NO mt+* gametes, (a) before deflagellation and (b) after 60 min of regeneration in the presence of cycloheximide.

its ultrastructure is highly ordered, with identical elements repeating numerous times from base to tip, it is in some ways valid to think of the organelle as a polymer, with assembly permitted only at the ends. In fact, what little experimental evidence exists suggests that most axoneme assembly occurs at the distal tip (21), although some assembly at both ends has by no means been ruled out. Standard kinetic theory for polymers predicts that growth of a given chain (or, in this case, of an entire axoneme) should occur as long as the subunit concentration is above some critical concentration (18). Given this context, it is easy to imagine that flagellar length is controlled by way of the precursor pool, with addition of subunits to the pool leading to flagellar growth, removal of subunits leading to flagellar resorption, and neither addition nor removal leading to the maintenance of flagellar length as it is.

Let us consider some predictions of a model in which the pool concentration of some flagellar structural component normally limits the extent of flagellar growth in each cell. We shall operationally measure the pool in terms of the extent of flagellar regeneration that occurs in the presence of the protein synthesis inhibitor cycloheximide. More will be said shortly about the validity of this definition. Further, we shall assume that pool "volumes" are the same for cells of identical size. This must be an assumption because there is no experimental evidence presently available on the physical location of the pool within the cell. What, then, does the model predict about the pool sizes of two equal-sized cells, one of which has three flagella of normal length and the other of which has one? The prediction is straightforward: both cells' pools should be the same. Our results, however, differ radically from this prediction. As demonstrated in Fig. 3, *vfl-2-220* cells with three flagella have pools three times as large as those with one flagellum, because their three flagella regenerate as far in cycloheximide as do the single flagella of the monoflagellates. We must conclude that total pool size, and probably also total pool concentration, does not determine flagellar length.

In fact, the results shown in Fig. 3 indicate that pool size may be simply proportional to flagellar number, or perhaps to total flagellar mass. We can imagine a number of models to account for this, but in none of them is flagellar assembly controlled by the pool size or concentration, and so we prefer not to speculate on the subject in this communication.

Several models for flagellar length determination have been proposed which hypothesize that the flagellar assembly process is intrinsically more difficult, and therefore slower, at longer flagellar lengths (1, 14). These models have the virtue of accounting for the well-established fact that flagellar regener-

ation kinetics are deceleratory. However, recent experiments from our laboratory using a short-flagella mutant of *C. reinhardtii* suggest that these models are inadequate to explain length regulation (7). The results presented here also argue against a model of the sort just mentioned. It could be imagined that, if assembly of longer flagella is intrinsically less favorable than assembly of shorter flagella, flagellar length would be proportional to the size or concentration of the pool. If this were the case, then monoflagellate *vfl-2* cells, whose pools are only one-third the size of those of trichoflagellate cells, should have considerably shorter flagella. In fact, they do not.

### The Extent of Regeneration in Cycloheximide Really Indicates the Quantity of Some Limiting Component

We have presented evidence here (Table II) indicating that the premature cessation of flagellar growth characteristic of regeneration in cycloheximide represents the exhaustion of some pool component. In the absence of new protein synthesis, cells regenerate to about one-half length. If deflagellated again, they are incapable of a second regeneration, as though some limiting component was used up during the first. If, on the other hand, the second deflagellation occurs when the flagella have grown to only one-quarter length, they do regenerate a second time, but now to a final length of one-quarter. These results are exactly what is expected if there is a pool that limits the extent of flagellar growth, because  $\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$ . The results are not consistent with an alternative possibility: that in the absence of protein synthesis the usual length determining system operates, but that for unknown reasons it regulates length to an abnormal, short value. If this were the case, then the flagella should again grow to one-half length; in fact, they do not. The results also indicate that the length "regulation" observed in the presence of cycloheximide is not analogous to that which occurs when protein synthesis is allowed, because under those more normal conditions, pool exhaustion clearly does not limit flagellar growth (21).

Although the extent of regeneration in the absence of new protein synthesis appears to reflect the amount of some limiting protein, or proteins, in the pool, it obviously says nothing about the specific identity of the protein(s). Indeed, we know of no data that point to any particular protein as being limiting, nor is it known whether one, or many, of the flagellar proteins exist in the pool in potentially limiting amounts.

### No Simple Relation Exists Between Cell Size, Flagellar Length, and Pool Size

Additional evidence that flagellar length is not determined by pool size comes from measurements of cell size, flagellar length, and pool size for cells of different sizes, ploidies, and states of differentiation (Table III). In summary, we find no simple relation between the three quantities. For example, small haploid gametes (50–150  $\mu\text{m}^3$  cell volumes) have 25.6  $\mu\text{m}$  of flagella (2 flagella, each 12.8  $\mu\text{m}$  long) and 7  $\mu\text{m}$  of pool ( $2 \times 3.5 \mu\text{m}$ ) whereas diploid gametes of the same size have 22.8  $\mu\text{m}$  of flagella and 10.6  $\mu\text{m}$  of pool. Thus, the cells with the longer flagella have the smaller pools. In contrast, large (450–550  $\mu\text{m}^3$ ) haploid vegetative cells have 22.4  $\mu\text{m}$  of flagella and 13  $\mu\text{m}$  of pool, whereas diploid vegetative cells of the same size have 24.2  $\mu\text{m}$  of flagella and 14.4  $\mu\text{m}$  of pool. Here the cells with the longer flagella also have the larger pools. Although none of the differences observed is dramatic, we feel that these

data provide some support for the notion that pool size does not determine flagellar length.

### The Relation of *vfl-2* to Other Known Genes Influencing Flagellar Number

As was mentioned in Results, a mutation conferring variable flagellar number was isolated by Adams and Luck and given the name *vfl-1*. This mutation was mapped to the right arm of linkage group VII, three map units from the centromere. The mutation described here, *vfl-2-220*, is unlinked to its centromere, and so it is not an allele of *vfl-1*. In addition, the phenotypes of the two differ. The flagellar number distribution in *vfl-1* cultures closely fits a poisson distribution (M. Adams, personal communication), whereas the distribution in a *vfl-2* culture clearly does not (Fig. 1). A few other reports of *Chlamydomonas* mutants with abnormal flagellar number exist (15, 24), but these mutants differ dramatically from *vfl-2* in phenotype in that their primary defects appear to be in cell division, so that cultures contain large multinucleate, multiflagellate cells. *Vfl-2* cells, in contrast, are normal in size, and, based on microscopic observations, they are mononucleate.

### Could the Pool be Compartmentalized

The existence of a flagellar precursor pool is well-established but nothing is known about its location. In interpreting the results reported in this paper, we have assumed, implicitly or explicitly, that pool volume is proportional to cell volume, so that a pool of a given size would be more concentrated in a small cell than in a large cell. If this is not the case, then, by definition, the pool (i.e., the length-limiting pool component(s), if they exist) must be compartmentalized. It could be, for example, that each flagellum has its own pool, so that a trirflagellate cell would have three times the pool size of a monoflagellate, while having an identical concentration of subunits in the pool. If such individual pools exist, however, their respective contents must be rather free to mix. Thus, when one of the two flagella of a cell is amputated, the remaining flagellum is resorbed as the lost one regenerates, and the resorbed material from the one is apparently used in the assembly of the other (21). Also, in many mutant/wild-type or mutant/mutant quadriflagellate dikaryons generated by mating, it has been demonstrated that protein from the pool donated by one parent appears in the flagella donated by the other (5, 19).

Support for this work was provided by grant PCM-7823253 from the National Science Foundation. J. W. Jarvik is the recipient of Research

Career Development Award KO4 AM00710 from the National Institutes of Health.

Received for publication 15 June 1981, and in revised form 24 August 1981.

### REFERENCES

- Child, F. M. 1978. The elongation of cilia and flagella: a model involving antagonistic growth zones. *ICN-UCLA Symp. Mol. Cell. Biol.* 12:351-358.
- Ebersold, W. T. 1967. *Chlamydomonas reinhardtii*: heterozygous diploid strains. *Science (Wash. D. C.)* 157:447-449.
- Fantes, P. A., W. D. Grant, R. H. Pritchard, P. E. Sudbery, and A. E. Wheals. 1975. The regulation of cell size and control of mitosis. *J. Theor. Biol.* 50:213-244.
- Hartwell, L. H., and M. W. Unger. 1977. Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. *J. Cell Biol.* 75:422-435.
- Huang, B., G. Piperno, Z. Rammanis, and D. J. L. Luck. 1981. Radial spokes of *Chlamydomonas* flagella. Genetic analysis of assembly and function. *J. Cell Biol.* 88:80-88.
- Jarvik, J. W., P. A. Lefebvre, and J. L. Rosenbaum. 1976. A cold-sensitive mutant of *Chlamydomonas reinhardtii* with aberrant control of flagellar length. *J. Cell Biol.* 70(2, Pt. 2):149 a (Abstr.).
- Jarvik, J., F. Reinhart, and S. Adler. 1980. Length control in the *Chlamydomonas* flagellum. *J. Cell Biol.* 87(2, Pt. 2):38 a (Abstr.).
- Jarvik, J. W., and J. L. Rosenbaum. 1980. Oversized flagellar membrane protein in paralyzed mutants of *Chlamydomonas reinhardtii*. *J. Cell Biol.* 85:258-272.
- Johnston, G. C., C. W. Ehrhardt, A. Lorinez, and B. L. A. Carter. 1979. Regulation of cell size in the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 137:1-5.
- Kates, J. R., and R. F. Jones. 1964. The control of gametic differentiation in liquid cultures of *Chlamydomonas*. *J. Cell. Comp. Physiol.* 63:157-164.
- Lefebvre, P. A., S. A. Nordstrom, J. E. Moulder, and J. L. Rosenbaum. 1978. Flagellar elongation and shortening in *Chlamydomonas* V. Effects of flagellar detachment, regeneration, and resorption on the induction of flagellar protein synthesis. *J. Cell Biol.* 78:8-26.
- Lefebvre, P. A., C. D. Silflow, E. D. Wieben, and J. L. Rosenbaum. 1980. Increased levels of mRNAs for tubulin and other flagellar proteins after amputation or shortening of *Chlamydomonas* flagella. *Cell* 20:469-477.
- Levine, R. P., and W. T. Ebersold. 1960. The genetics and cytology of *Chlamydomonas*. *Annu. Rev. Microbiol.* 14:197-216.
- Levy, E. M. 1974. Flagellar elongation: an example of controlled growth. *J. Theor. Biol.* 43:133-149.
- Lewin, R. A. 1952. Ultraviolet induced mutations in *Chlamydomonas moewusii* gerolf. *J. Gen. Microbiol.* 6:233-248.
- Lewin, R. A. 1954. Mutants of *Chlamydomonas moewusii* with impaired motility. *J. Gen. Microbiol.* 11:358-369.
- Mortimer, R. K., and D. C. Hawthorne. 1966. Genetic mapping in saccharomyces. *Genetics* 53:165-173.
- Oosawa, F., and M. Kasai. 1962. A theory of linear and helical aggregation of macromolecules. *J. Mol. Biol.* 4:10-21.
- Piperno, G., B. Huang, and D. J. L. Luck. 1977. Two-dimensional analysis of flagellar proteins from wild-type and paralyzed mutants of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* 74:1600-1604.
- Prescott, D. M. 1956. Relation between cell growth and cell division. III. Changes in nuclear volume and growth rate and prevention of cell division in *Amoeba proteus* resulting from cytoplasmic amputations. *Exp. Cell Res.* 11:94-98.
- Rosenbaum, J. L., J. F. Moulder, and D. L. Ringo. 1969. Flagellar elongation and shortening in *Chlamydomonas*. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* 41:600-619.
- Sager, R., and S. Granick. 1953. Nutritional studies with *Chlamydomonas reinhardtii*. *Ann. N. Y. Acad. Sci.* 56:831-838.
- Starling, D., and J. Randall. 1971. The flagella of temporary dikaryons of *Chlamydomonas reinhardtii*. *Genet. Res.* 18:107-113.
- Warr, J. R. 1968. A mutant of *Chlamydomonas reinhardtii* with abnormal cell division. *J. Gen. Microbiol.* 52:243-251.
- Weeks, D. P., and D. S. Collis. 1976. Induction of microtubule protein synthesis in *Chlamydomonas reinhardtii* during flagellar regeneration. *Cell* 9:15-27.
- Ycas, M., M. Sugita, and A. Bensam. 1965. A model of cell size regulation. *J. Theor. Biol.* 9:444-470.