FLAGELLAR ELONGATION AND SHORTENING IN *CHLAMYDOMONAS*

II. Re-utilization of Flagellar Proteins

BRIAN COYNE and JOEL L. ROSENBAUM. From the Department of Biology, Yale University, New Haven, Connecticut 06520

Regeneration of flagella amputated from the biflagellate *Chlamydomonas* depends in part on an available pool of flagellar precursor protein and in part on new protein synthesis (10). Observations on the regeneration kinetics in populations and single cells of *Chlamydomonas* pf 16 (paralyzed flagella) revealed that when both flagella are removed (the "zero-zero" case), both regenerate to a length of 10–12 μ. In the presence of a concentration of cycloheximide adequate to inhibit amino acid incorporation into protein by at least 99% (10 μg/ml), however, regeneration is limited to about 2 μ per flagellum. From these results, it was concluded that the cells have a limited amount of the flagellar precursor protein necessary for regeneration (10).

In single cells, it was observed that if only one of the two flagella is removed (the "long-zero" case), the intact flagellum initially shortens by linear kinetics while the amputated flagellum regenerates. This simultaneous shortening and elongation continues until both flagella are of

approximately equal length, whereupon the two flagella elongate together by deceleratory kinetics. In the absence of protein synthesis, the pattern of events is similar to that occurring in uninhibited long-zero cells: The intact flagellum still shortens and the amputated flagellum elongates, and when they reach the same length they elongate together. Of particular interest here was the observation that in the absence of protein synthesis the total length of new flagellum formed in the long-zero cells was considerably greater than that formed in inhibited cells with *both* flagella removed. This result suggested that the additional flagellar growth in cycloheximide-inhibited long-zero cells might be occurring by re-utilization of flagellar proteins from the shortening flagellum. If this were so, then, for long-zero cells in the presence of cycloheximide, the greater the *initial* length of the intact flagellum, the greater should be the flagellar length subsequently attained by regeneration. Moreover, for each additional micron of intact flagellum initially present in a long-zero cell, there should be one-half micron added to the *final length* of *each* flagellum if, in fact, the flagellar precursor protein is being divided equally between the two flagella. Measurements reported here of the *final* flagellar lengths of single cells for a variety of *initial* lengths of the intact flagellum of long-zero cells in the presence of cycloheximide are in excellent agreement with this prediction, and support the conclusion that flagellar proteins from the shortening flagellum are re-utilized for the subsequent flagellar regeneration.

MATERIALS AND METHODS

Cultures

Strain pf 16 of *Chlamydomonas* was grown under synchronous conditions as described previously (10). The flagella of this strain are paralyzed, but the 9 + 2 microtubule structure appears normal in electron micrographs (Ringo and Rosenbaum, unpublished data).

Flagella Amputation

Cells having one flagellum removed and one intact (long-zero cells) or both flagella removed (zero-zero cells) were obtained as previously described (10).

Inhibition of Protein Synthesis

To inhibit protein synthesis, cycloheximide was added to the culture at a concentration of 10 μg/ml just prior to amputation. Previous work (10) has shown that this concentration completely and immediately inhibits amino acid incorporation into trichloroacetic acid-precipitable protein of *Chlamydomonas.*

Measurement of Regeneration in Single Cells

With the techniques previously described (10), a series of phase-contrast photomicrographs of the regenerating flagella of single cells was made at various times after amputation. The photographic negatives were projected at a standard enlargement, tracings were made of the projected flagella, and these tracings were measured.

The average flagellar length over the time interval 60–90 min was used as an estimate of final flagellar length. For long-zero cells, the initial length of the intact flagellum was determined by extrapolating the linear resorption curve of the intact flagellum backwards to time zero. This was necessary since there was always a 2–3 min delay between amputation and the first photographic exposure, during which time the intact flagellum had already started shortening.

RESULTS

Zero-zero Regeneration

Fig. 1 *a* is the regeneration curve obtained from a single cell of strain pf 16 after removal of both flagella. Regeneration proceeds with no notice-

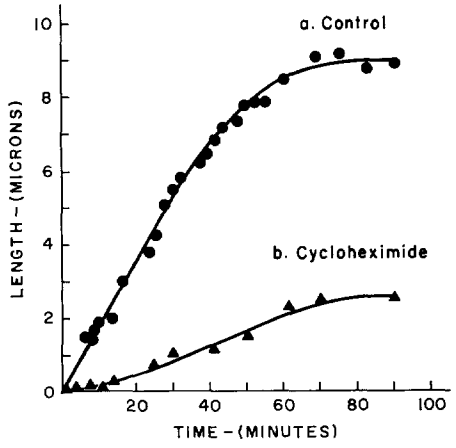

FIGURE 1 Curve *a*: The kinetics of flagellar regeneration in a single cell of *Chlamydomonas* (pf 16) following removal of both flagella (closed circles). Curve *b*: Effect of cycloheximide (10 μg/ml) on flagellar regeneration in a single cell of *Chlamydomonas* (pf 16) following removal of both flagella (closed triangles).

able lag and decelerates. Most growth is completed by about 90 min, but some growth continues at a slow rate thereafter (10). Similar regeneration curves for populations of cells of wild type strains of *Chlamydomonas* (3, 8, 10), as well as for other flagellated protozoans, have been reported previously (9, 11).

The same experiment performed on a single cell when protein synthesis was inhibited by cycloheximide (10 μg/ml) is illustrated in Fig. 1 *b*. In this case, elongation of each flagellum was limited to 2.5 μ. Observations of 47 single cells yielded an average final length per flagellum of 2.6 μ (SD = 1.1 μ per flagellum).

Long-zero Regeneration

Fig. 2 *a* illustrates the simultaneous shortening and elongation of the flagella of a single long-zero cell. When the regenerating and the receding intact flagella attain a common intermediate length, the shortening process is halted and both flagella elongate together. Occasionally, however, the intact flagellum continues to shorten after the flagellar lengths become equal, as in Fig. 2 *b*. This phenomenon will be referred to as "undershoot." In this case, as the resorbing flagellum continues to shorten, the elongating flagellum grows slowly or not at all until the intact flagellum stops shortening and regenerates to the same length as the "waiting" flagellum. Both flagella then elongate together.

In the presence of cycloheximide, most long-zero cells displayed the pattern of shortening and elongation shown in Fig. 2 *a*, the undershoot being rare and *almost always confined to those experiments in which the initial length of the intact flagellum was long (10 μ or more)*.

Fig. 3 illustrates how regeneration of long-zero cells in cycloheximide depends on the initial length of the intact flagellum. Comparing Fig. 3 *a*, *b*, and *c*, it is seen that *as the initial length of the intact flagellum is decreased, both the rate and degree of shortening of the intact flagellum, as well as the final combined length of the regenerated flagella, are diminished.* These results indicate that, in the absence of protein synthesis, long-zero cells may regenerate two flagella by drawing protein from two sources — the intact flagellum, and the pool of flagellar protein precursors within the cell itself. This can be described mathematically as follows: Let I be the initial length of the intact flagellum, and P the total length of flagellum that can be constructed

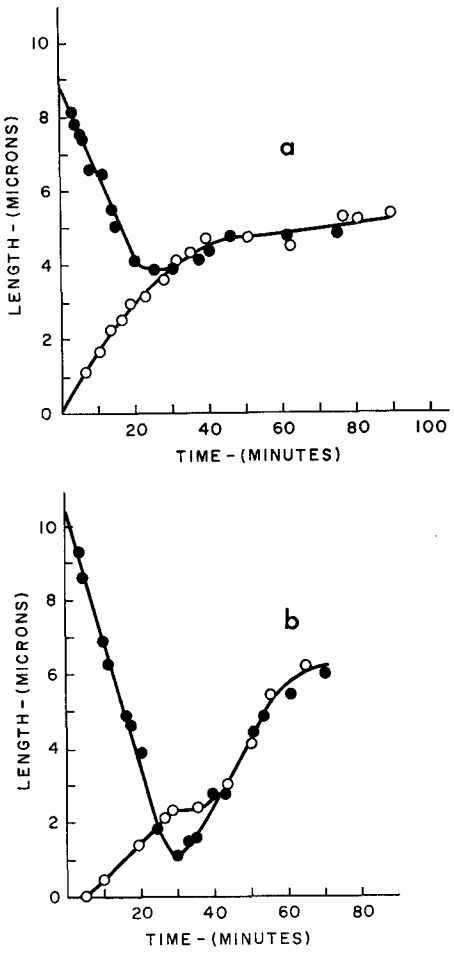

FIGURE 2 The kinetics of flagellar shortening and elongation in a single long-zero *Chlamydomonas* (pf 16) showing (*a*) partial shortening of the intact flagellum and (*b*) almost complete shortening ("undershoot") of the intact flagellum and the lag in the regeneration of the amputated flagellum. Open circles, amputated flagellum; closed circles, intact flagellum.

using only the flagellar precursors of the cell pool. By the use of all the flagellar precursors available to it, the total length of flagellum that a long-zero cell can construct in the absence of protein synthesis is I plus P, provided that the cell can reutilize the precursor proteins in the resorbing intact flagellum. If the total regenerated flagellar growth ($I + P$) is evenly divided between the two flagella, then the final length of each flagellum obeys the relation

$$F = \tfrac{1}{2}(I + P).$$

When F is plotted versus I, this theoretical relation describes a straight line with slope 0.5 and intercept $P/2$, where $P/2$ is the final flagellar length of zero-zero cells (for which I is zero). As noted previously, our data show that each flagellum of zero-zero cells regenerates to 2.6 ± 1.1 μ. In Fig. 4, the theoretical line with slope 0.5 and intercept 2.6 μ is plotted. The dashed lines with slope 0.5 and intercepts 1.5 and 3.7 μ (± 1 SD) define a corridor within which should lie most data for long-zero cells with various initial lengths of intact flagella which have been allowed to regenerate in the absence of protein synthesis. Data so plotted in Fig. 4 fall largely within this corridor. Furthermore, linear least squares analysis of the data from 18 long-zero cells yields a line with slope 0.503 and intercept 2.03 μ, which compares favorably with the predicted slope 0.5 and the experimentally determined intercept 2.6 ± 1.1 μ.

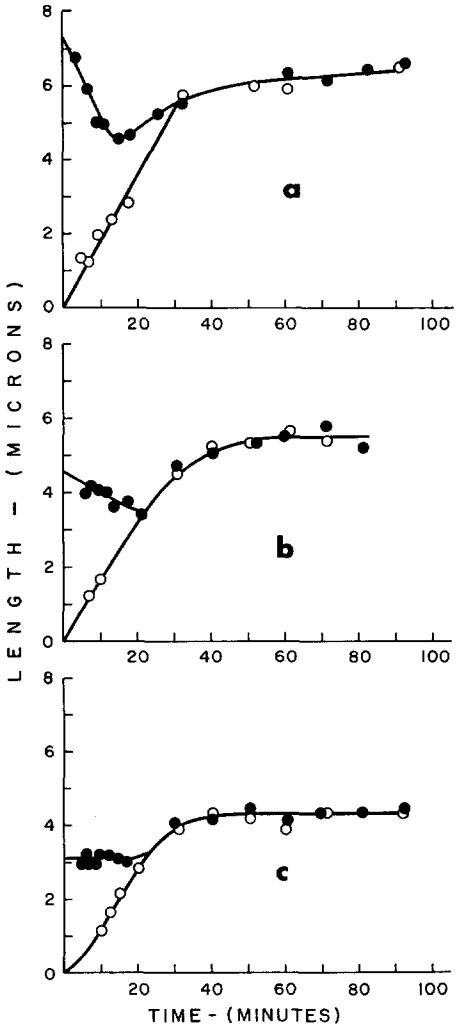

FIGURE 3 Dependence of the kinetics of flagellar shortening and elongation and final flagellar length on the initial length of the intact flagellum. Initial length of the intact flagellum was (*a*) 7.2 μ; (*b*) 5.1 μ; (*c*) 3.1 μ. Open circles, amputated flagellum; closed circles, intact flagellum. All experiments in the presence of cycloheximide (10 μg/ml).

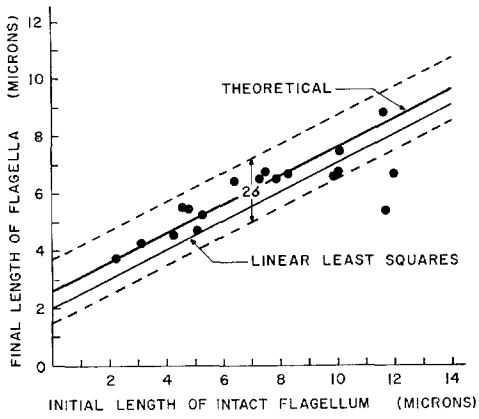

FIGURE 4 Final regenerated length of flagella plotted versus the initial length of the intact flagellum for long-zero *Chlamydomonas* (pf 16) in the presence of cycloheximide (10 μg/ml). Each closed circle represents measurements on a single cell. Also plotted is the theoretical relation $F = \frac{1}{2}(I + P)$ as a straight line with slope 0.5 and intercept $P/2 = 2.6$ μ. The dashed lines represent the limits of variation (±1 SD) of this theoretical relation for $P/2 = 2.6 \pm 1.1$ μ. The linear least squares line is plotted with slope 0.503 and intercept 2.03 μ.

DISCUSSION

The various advantages of the *Chlamydomonas* flagella-regenerating system for investigation of the control of organelle development have been described previously (10). Large quantities of cells can be cultured in synchrony (1, 5), the flagella are easily removed and fractionated (Witman, Carlson, and Rosenbaum, in preparation), and many flagellar mutants are available for genetic analysis (Coyne and Rosenbaum, unpublished data; 6, 7, 12).

An added attraction of this regenerating system in *Chlamydomonas* is the fact that the organism is biflagellate. This affords an opportunity to observe the interactions between parts of the system when one of the two flagella is removed. An earlier

report (10) showed that removal of one flagellum is followed immediately by shortening of the intact flagellum and simultaneous regeneration of the amputated flagellum; at some common intermediate length the two flagella grow out together. The experiments reported here show that proteins from the resorbing flagellum are conserved and re-utilized as such in the formation of new flagella.

Although this work does not indicate which of the flagellar proteins are resorbed and re-utilized (membrane, matrix or microtubule), previous work has suggested that at least the microtubule proteins can be accumulated and re-utilized at a later time to form flagella in the absence of protein synthesis (10). Thus, when deflagellated *Chlamydomonas* are treated with colchicine, no flagellar growth occurs, but protein synthesis remains normal. It would appear that in this situation the cells are still making and accumulating microtubule proteins, since release from colchicine inhibition coupled with simultaneous inhibition of any new protein synthesis with cycloheximide results in the formation of more flagellum than if the cells were not pretreated with colchicine (10). By the use of this colchicine treatment of deflagellated cells, we have now been able to obtain the formation of almost complete flagella in the absence of protein synthesis (Moulder and Rosenbaum, unpublished results). Assuming that the effect of colchicine is to bind microtubule protein (2), these results indicate that microtubule protein is accumulated in colchicine-blocked deflagellated *Chlamydomonas*, and that this microtubule protein can be utilized at a later time to form flagella. Such experiments on the accumulation of microtubule protein combined with the results described in this report suggest the possibility that cells having microtubular organelles can resorb these structures and re-utilize the microtubular subunits for assembling microtubules of the same organelles (or perhaps even of different organelles) at some other time in the cell cycle. In fact, there is good evidence that many flagellated protozoans, including *Chlamydomonas*, normally resorb their flagella prior to cytokinesis (4, 7, and see reference 11 for review), and it is not unlikely that the resorbed flagellar microtubule proteins are conserved and utilized in the formation of daughter cell flagella. The results presented here show quite clearly that such re-utilization of flagellar proteins does occur in the subsequent formation of the same organelle.

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