BMC Cell Biology

BioMed Central

Research article

Open Access

Chlamydomonas fla mutants reveal a link between deflagellation and intraflagellar transport

Jeremy David Kirk Parker and Lynne Marie Quarmby*

Address: Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada

Email: Jeremy David Kirk Parker - jdparker@sfu.ca; Lynne Marie Quarmby* - quarmby@sfu.ca

* Corresponding author

Published: 20 August 2003

BMC Cell Biology 2003, **4**:11

Received: 27 June 2003

Accepted: 20 August 2003

This article is available from: http://www.biomedcentral.com/1471-2121/4/11

Abstract

Background: Cilia and flagella are often lost in anticipation of mitosis or in response to stress. There are two ways that a cell can lose its flagella: resorption or deflagellation. Deflagellation involves active severing of the axoneme at the base of the flagellum; this process is defective in *Chlamydomonas fa* mutants. In contrast, resorption has been thought to occur as a consequence of constitutive disassembly at the tip in the absence of continued assembly, which requires intraflagellar transport (IFT). *Chlamydomonas fla* mutants are unable to build and maintain flagella due to defects in IFT.

Results: *fla10* cells, which are defective in kinesin-II, the anterograde IFT motor, resorb their flagella at the restrictive temperature (33°C), as previously reported. We find that in standard media containing ~300 microM calcium, *fla10* cells lose flagella by deflagellation at 33°C. This temperature-induced deflagellation of a *fla* mutant is not predicted by the IFT-based model for flagellar length control. Other *fla* mutants behave similarly, losing their flagella by deflagellation instead of resorption, if adequate calcium is available. These data suggest a new model whereby flagellar resorption involves active disassembly at the base of the flagellum via a mechanism with components in common with the severing machinery of deflagellation. As predicted by this model, we discovered that deflagellation stimuli induce resorption if deflagellation is blocked either by mutation in a *FA* gene or by lack of calcium. Further support for this model comes from our discovery that *fla10-fa* double mutants resorb their flagella more slowly than *fla10* mutants.

Conclusions: Deflagellation of the *fla10* mutant at the restrictive temperature is indicative of an active disassembly signal, which can manifest as either resorption or deflagellation. We propose that when IFT is halted by either an inactivating mutation or a cellular signal, active flagellar disassembly is initiated. This active disassembly is distinct from the constitutive disassembly which plays a role in flagellar length control.

Background

Intraflagellar transport (IFT) was first characterized in the unicellular green alga *Chlamydomonas* [1] and has since been shown to be required for flagellar assembly in a variety of systems [2,3]. IFT is the bidirectional movement of large protein complexes (IFT particles) along the flagellar axoneme, and has anterograde and retrograde components mediated by the plus and minus-end directed microtubule motors kinesin-II and cytoplasmic dynein, respectively [reviewed in [4,5]]. In *Chlamydomonas*, null

mutations in genes necessary for activity of either kinesin-II or cytoplasmic dynein result in bald (flagella-less) cells or cells with very short, abnormal flagella [6,7]. Retrograde IFT is not proposed to be directly involved in disassembly, but rather is necessary to recycle IFT particles [7,8].

A model for flagellar length control has been proposed wherein anterograde IFT is required for transport of axonemal precursors to the distal tip of the flagellum; these precursors are necessary both for *de novo* flagellar assembly and to offset the constitutive disassembly that occurs at the tips of flagella [8]. This model suggests that the steady-state length of a flagellum is determined kinetically by the relative contributions of assembly, mediated by anterograde IFT, and disassembly at the tip, which is IFT-independent [9]. Thus, the phenotype of *Chlamydomonas* long flagella mutants could be a result of either an upregulation of anterograde IFT, or due to a decrease in the rate of disassembly at the tip [8–10].

A *Chlamydomonas* temperature-sensitive mutant for flagellar assembly, *fla10*, has been characterized as having a lesion in a subunit of kinesin-II [11]. *fla10* cells have wild-type flagella at the permissive temperature (20°C), but are bald at the restrictive temperature (33°C) [12]. In agreement with the length control model, *fla10* cells incubated at an intermediate temperature have intermediate-length flagella [8]. It has been accepted that the flagella of *fla10* cells resorb at the restrictive temperature due to continued disassembly in the absence of anterograde IFT [e.g., [13]].

Several other temperature-sensitive flagellar assembly mutants (*fla* mutants) are available in *Chlamydomonas* [12,14,15]. Unlike *fla10*, the genes for these mutants have not yet been identified. However, like *fla10*, these *fla* mutants have been shown to have defects in IFT, even at the permissive temperature [15,16]. The majority of the *fla* mutants have been reported to undergo flagellar resorption at 33°C [12,15,16], presumably due to defects at different points in the IFT cycle disrupting flagellar assembly. Most *fla* mutants are unable to regenerate flagella at 33°C, with the exception of *fla2* [12]. *fla2* is also exceptional in that it has been observed to deflagellate, rather than resorb, at the restrictive temperature [2,12,14].

Deflagellation, like IFT, is a conserved process in eukaryotic cilia and flagella [reviewed in [17]. Deflagellation/deciliation is the regulated severing of the axoneme, and has been shown to occur in response to a number of stimuli, including pH shock, 42°C heat shock, and treatment with dibucaine or alcian blue. Calcium plays a central role in signalling deflagellation: calcium influx mediates acid shock-induced deflagellation [18], and axonemal severing can be induced *in vitro* in response to calcium [19]. Our lab has cloned two essential components of the deflagellation pathway, the flagellar autotomy genes *fa1* and *fa2* [20,21]. *fa* mutants do not undergo deflagellation in response to any known stimulus. We have also demonstrated that the microtubule-severing ATPase katanin is likely to mediate axonemal severing during deflagellation [19,22]. We have localized both katanin and Fa1p to the site of deflagellation, the flagellar transition zone between the basal body and the flagellum proper [[20], M. Mahjoub and LMQ, unpublished observations].

In many ciliated cells, including vertebrate cells and *Chlamydomonas*, flagella/cilia are shed or resorbed prior to mitosis [23–26]. It has been proposed that mammalian primary cilia are important to maintain cells in a differentiated state; the best-characterized example is the role of kidney epithelial cilia in models of polycystic kidney disease [reviewed in [27]]. Therefore, as has already been demonstrated for flagellar assembly [3,27], genes required for flagellar resorption or deflagellation may be implicated in disease and/or development.

It is unknown what determines whether a given cell type deflagellates or resorbs prior to mitosis. Indeed, even in the same organism, flagellar loss may occur via different mechanisms at different stages of the life cycle [23]. Flagellar resorption and deflagellation have been thought to occur by dramatically different mechanisms and at different locations: resorption via disassembly at the tip [8,13] and deflagellation by severing at the base [28]. However, a role for severing activity at the base during resorption has been suggested by elegant EM studies performed on cells resorbing their flagella prior to mitosis [29]. We now provide further evidence that flagellar resorption involves severing activity at the base, and that active disassembly by resorption is distinct from the constitutive disassembly involved in flagellar length control.

Our examinations of the phenotypes of *fla10* and *fla2*, reported here, lead us to the conclusion that flagellar resorption of *fla* mutants results from active disassembly, rather than constitutive disassembly in the absence of IFT. We show that in common culture medium for *Chlamydomonas*, the predominant mode of flagellar loss at 33°C for *fla10* is deflagellation. Blocking the ability to deflagellate, either by lowering the extracellular calcium concentration or by genetically blocking the deflagellation pathway, causes *fla10* cells to resorb their flagella at the restrictive temperature. Likewise, we demonstrate that *fla2* cells undergo flagellar loss via deflagellation, and that resorption occurs in low calcium or by genetically introducing a *fa* mutation. Furthermore, *fla10-fa1* and *fla10-fa2* double mutant populations are defective for resorption, as many cells retain flagella past the time when *fla10* mutants are bald. Finally, we find that flagellar resorption

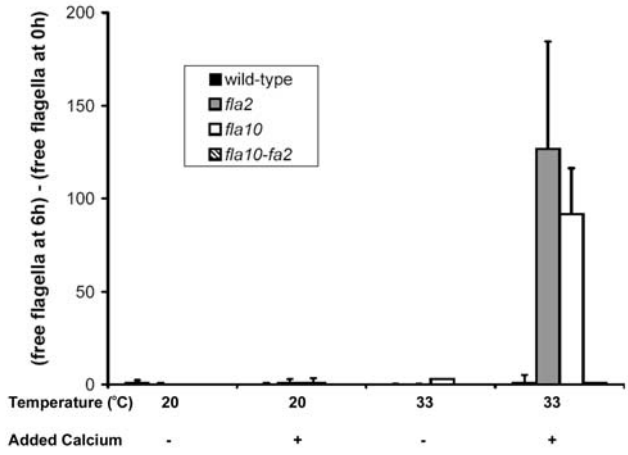

Figure 1
Free flagella in *Chlamydomonas* media are indicative of deflagellation. Determination of free flagella under various conditions. Free flagella observed per 100 cells were counted after 0 and 6 hours at 20°C or 33°C, in either the presence (TAP medium or HEPES+Ca) or absence (HEPES buffer) of calcium, and the difference 6 h – 0 h was calculated. Each data point is the average of at least three experiments on independent cultures.

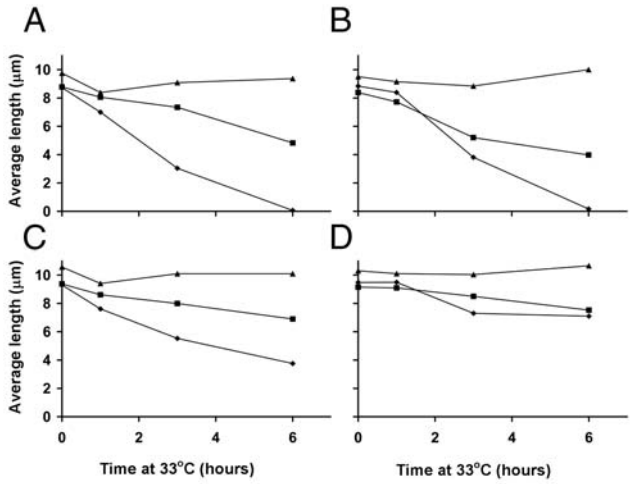

Figure 2
***fla10* and *fla2* average flagellar lengths decrease during incubation at 33°C.** Cells were resuspended in pre-warmed buffer at t = 0 h. At least 70 cells were examined per timepoint. ▲ wild-type, ■ *fla2*, ◆ *fla10*. (A) Incubation in HEPES, including contribution of bald and uniflagellate cells.(B) Incubation in HEPES+Ca, including contribution of bald and uniflagellate cells.(C) The same samples as (A), but averages exclude contributions of zero-length flagella.(D) The same samples as (B), but averages exclude contribution of zero-length flagella.

can be induced in cells unable to deflagellate in response to deflagellation stimuli. Our findings indicate that the deflagellation and resorption pathways are not separate, but that each can result from a disassembly signal which culminates at the flagellar transition zone. We propose that IFT and flagellar disassembly share common regulatory elements.

Results and Discussion

fla10 *and* fla2 *deflagellate at the restrictive temperature*

Cultures of *fla2* cells in TAP medium contain free flagella after incubation at the restrictive temperature of 33°C. Due to their small size, and tendency to stick to each other and to attached flagella, free flagella are difficult to accurately identify; however, as *fla2* cells can regenerate and re-deflagellate at 33°C, the appearance of abundant free flagella is quite obvious. Our initial observations of the *fla10* temperature-sensitive phenotype led us directly to the conclusion that this mutant also deflagellates, as many free flagella are seen in the presence of calcium at 33°C in both *fla10* and *fla2* cultures (Figure 1).

As *fla10* has only been previously characterized as resorbing flagella at 33°C, and given our knowledge of the role played by calcium in the deflagellation pathway, we reasoned that deflagellation would be prevented if *fla10* cells were incubated at 33°C in buffer lacking added calcium. We found this indeed to be the case; there is no indication of deflagellation by *fla10* cells at 33°C in the absence of calcium, yet cultures are still mostly bald after ~6 hours (Figures 1, 2). We extended our observations by demonstrating that *fla2* cells also do not deflagellate at 33°C in the absence of calcium (Figure 1). Adding calcium to minimal buffer to a concentration approximately that of TAP media (340 μM) [30] restored the deflagellation phenotype of both *fla* mutants. As a further control, blocking the deflagellation response by creating double mutants of either *fla10* or *fla2* with either of *fa1* or *fa2* also prevented the appearance of free flagella after six hours (Figure 1 and data not shown). Wild-type cells do not deflagellate under these conditions.

Having examined the culture media under various conditions, we turned our attention to the cells themselves. The average flagellar lengths of *fla10* and *fla2* cells, but not wild-type cells, decrease at 33°C (Figure 2). We have shown that the *fla* mutants deflagellate at 33°C in the presence of added calcium, yet the differences in the average flagellar length plots are difficult to discern whether or not calcium was added, especially for *fla10* (diamonds in Figure 2A,2B). This similarity of the average flagellar

length plots holds whether or not zero-length flagella are included in the average length calculations (Figure 2C,2D).

To better illustrate the effects of calcium at the restrictive temperature on the *fla* mutants, we have chosen a novel means of presenting flagellar length data. By plotting the length of the longer flagellum versus the length of the shorter flagellum for *fla10*, *fla2*, and wild-type cells (Figures 3, 4, and 5, respectively), we can more clearly observe the states of individual cells during timecourses at the restrictive temperature. Points representing bald cells fall at the origin, while points representing uniflagellate cells are found on the x-axis. Additionally, we have plotted the percent of biflagellate, uniflagellate, and bald cells in the population for each timepoint as insets in the scatter plots. This presentation allows one to quickly assess the flagellation state of a population, and changes in that state over time.

Figure 3 presents timecourses for *fla10* flagellar lengths at 33°C, in the absence or presence of added calcium. In both populations, cells begin with flagella of approximately equal lengths, and the distribution of flagellar lengths falls from ~8–11 μm. After one hour at the restrictive temperature, flagellar lengths of most cells in calcium remain unchanged, although a few cells now have flagella of unequal lengths. In contrast, the flagella of cells in calcium-free buffer have shortened to 5–10 μm (average length of 7.5 μm, Figure 2C).

After three hours at 33°C, cells in each population have shorter flagella, and there is an increase in the numbers of uniflagellate and bald cells in both populations. At this time in nominally calcium-free buffer, several cells with flagella shorter than 4 μm are observed. These short flagella indicate that resorption is occurring. Conversely, in the presence of calcium, no flagella <4 μm are seen, suggesting that cells deflagellate before flagellar resorption is complete. *fla10* cells incubated at 20°C did not undergo significant flagellar loss or shortening, and after 6 hours at 20°C, populations resembled zero-hour timepoints (bottom panels in Figure 3).

Figure 4 is a timecourse for the *fla2* mutant, which was previously reported to deflagellate at the restrictive temperature [2,12,14]. After 6 hours in HEPES at 33°C, but not at 20°C, over one-quarter of the *fla2* cells are bald. Fewer cells with flagella >9 μm are seen after 6 hours at 33°C compared with earlier timepoints and 20°C controls. At the three hour timepoint in the presence of calcium, there is a dramatic increase in bald cells in the *fla2* population (Figure 4, inset). In contrast to *fla10*, *fla2* cells are able to regenerate flagella at the restrictive temperature [[12,14], and data not shown]. This regeneration accounts for the increase in the number of cells with short flagella in the *fla2* population at later timepoints. After overnight incubation at 33°C, *fla2* cultures are often entirely bald.

Figure 5 shows the flagellar lengths of wild-type cells after a shift to 33°C. In contrast to the *fla* mutants, wild-type flagella do not undergo appreciable changes in length at 33°C. In principle, the resorption phenotype of *fla10* at 33°C could be solely due to constitutive disassembly in the absence of anterograde IFT, and the deflagellation phenotype a secondary consequence due to the stress of elevated temperature. However, wild-type cells do not deflagellate at 33°C (Figures 1, 5), although they will deflagellate at 42°C (data not shown).

fla10-fa *double mutants are slow to resorb flagella*

The deflagellation phenotype of *fla10* and *fla2* at 33°C in calcium is blocked by either *fa* mutation (Figure 1 and data not shown). We wished to examine double mutant cell populations, and because *fla2* is able to regenerate flagella at the restrictive temperature, we focused on *fla10-fa* mutants. We find that both *fla10-fa1* and *fla10-fa2* double mutants resorb flagella more slowly than *fla10* at 33°C (Figure 6). This held true whether or not calcium was present in the medium, and, as for the *fla10* single mutant, similar flagellar loss kinetics were observed regardless of calcium (Figure 3 and data not shown). This result is reminiscent of the partial block of *fla10* flagellar resorption by six different extragenic suppressors [31]; it is unknown whether any of these suppressors are *fa* genes.

fla10 mutant populations are >90% bald after 6 hours at 33°C (Figures 3, 6), whereas only ~1/3 of *fla10-fa* cells are bald at this time (Figure 6). Moreover, at later timepoints when *fla10* cells are all bald, many double mutant cells still retain flagella. Strikingly, at all timepoints, the double mutant populations are enriched in cells with unequal length flagella as well as uniflagellate cells. Resorbing populations of *fla10* cells also have a few uniflagellate cells, but cells with unequal length flagella are not often observed (Figure 3). Thus, the appearance of cells with flagella of unequal length in double mutant populations correlates with an overall decrease in the resorption rate.

The prevalence of uniflagellate cells, as well as flagella of unequal lengths, is at odds with the classical picture of *Chlamydomonas* long-zero flagellar dynamics [8,32]. This model predicts that cells with flagella of unequal length should be rarely seen: the combination of length-control kinetics, and the fact that the two flagella share a common pool of precursors, means that any length imbalance between the flagella of a single cell will be corrected [8]. However, long-zero experiments are typically carried out in conditions permissive for flagellar regeneration, which

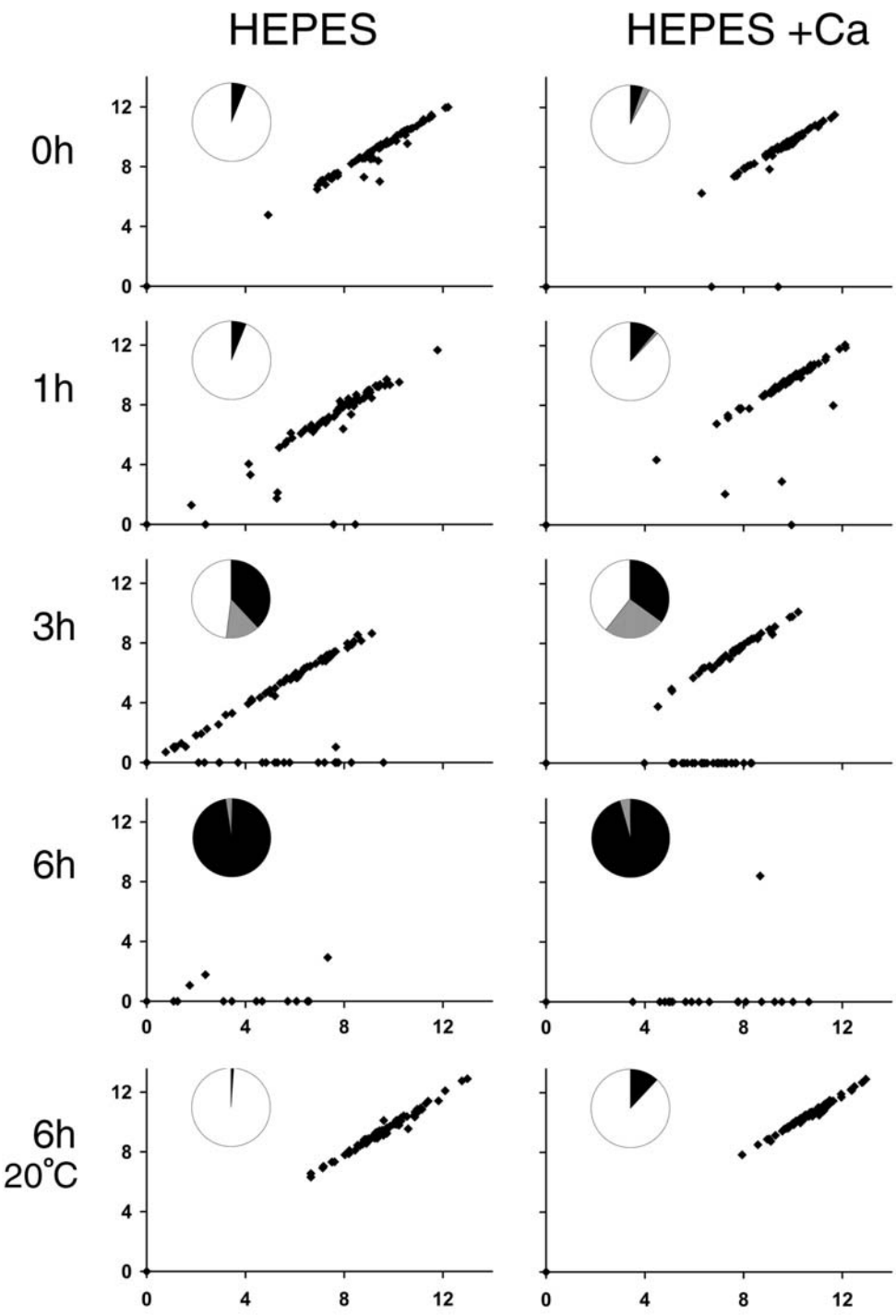

Figure 3
Scatter plots of *fla10* timecourses in HEPES and HEPES+Ca. Data shown is the same as *fla10* data in Figure 2. Each flagellum from at least 70 cells was measured, and the lengths plotted as the length of the longer flagellum (in μm) on the X-axis and the length of the shorter flagellum (in μm) on the Y-axis, such that each point in a graph corresponds to a single cell (except where two points overlap, such as at the origin). Insets show the percent of biflagellate (white), uniflagellate (grey), and bald (black) cells in each sample.

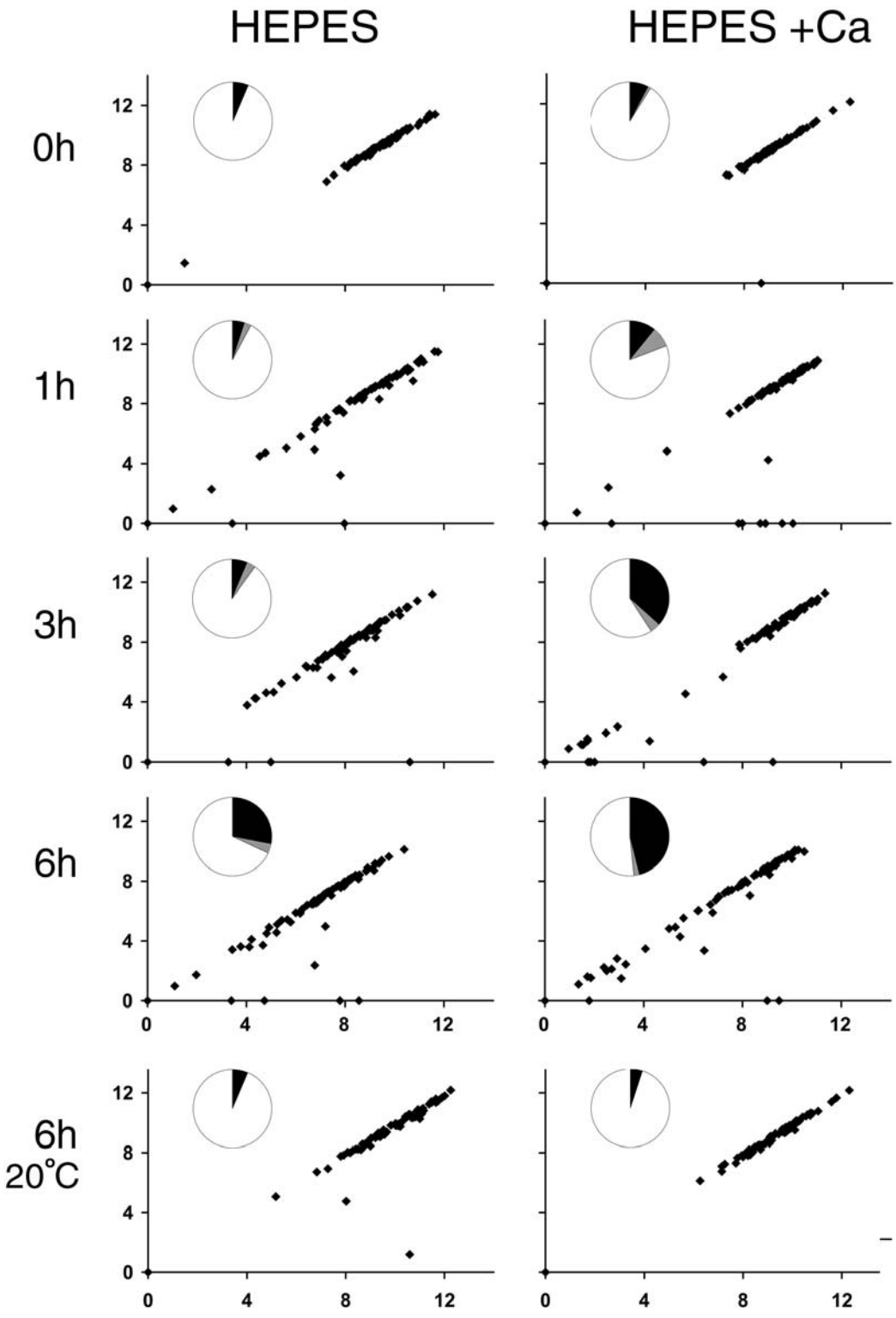

Figure 4
Scatter plots of *fla2* timecourses in HEPES and HEPES+Ca. See Figure 3 legend for further details.

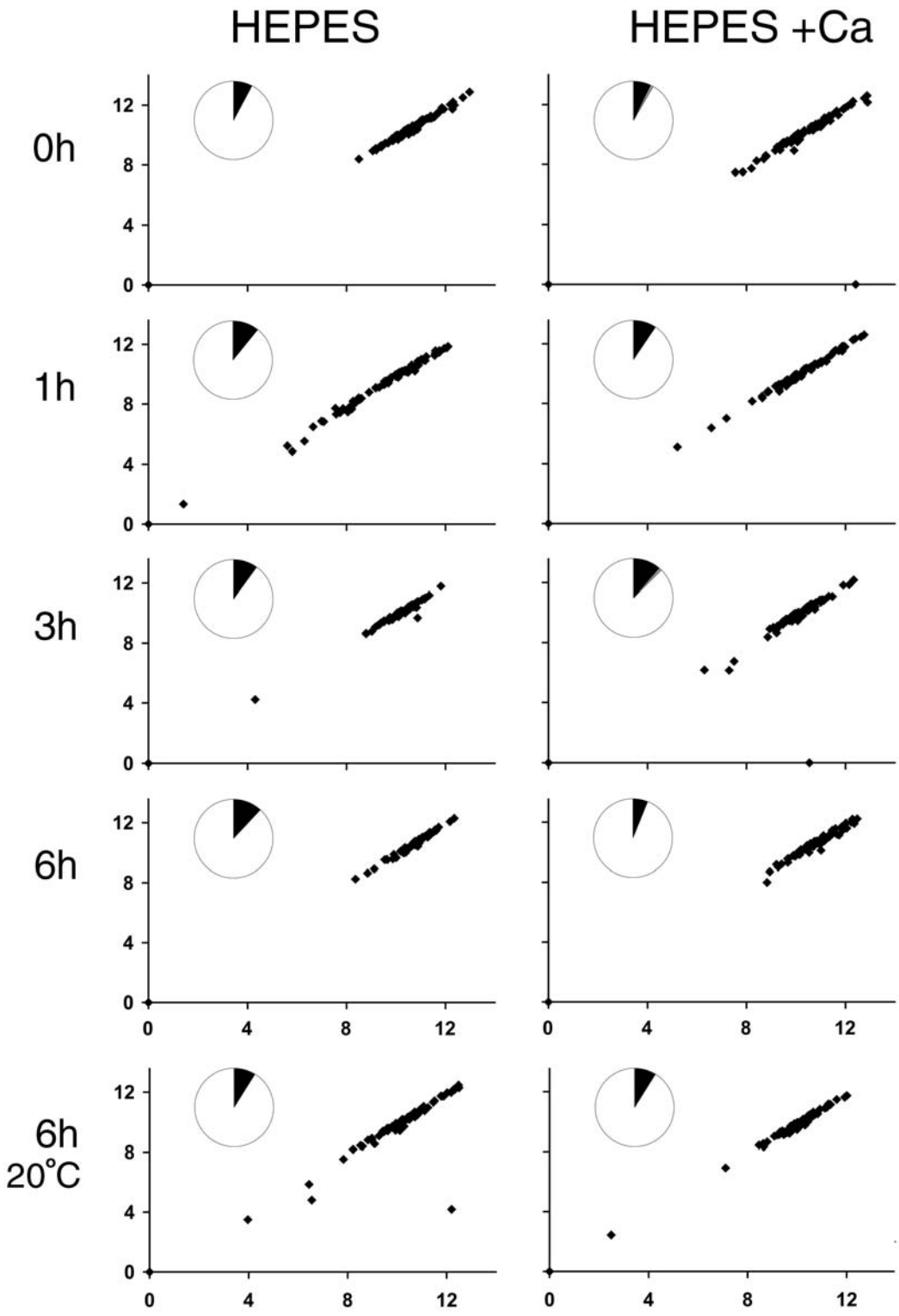

Figure 5
Scatter plots of wild-type timecourses in HEPES and HEPES+Ca. See Figure 3 legend for further details.

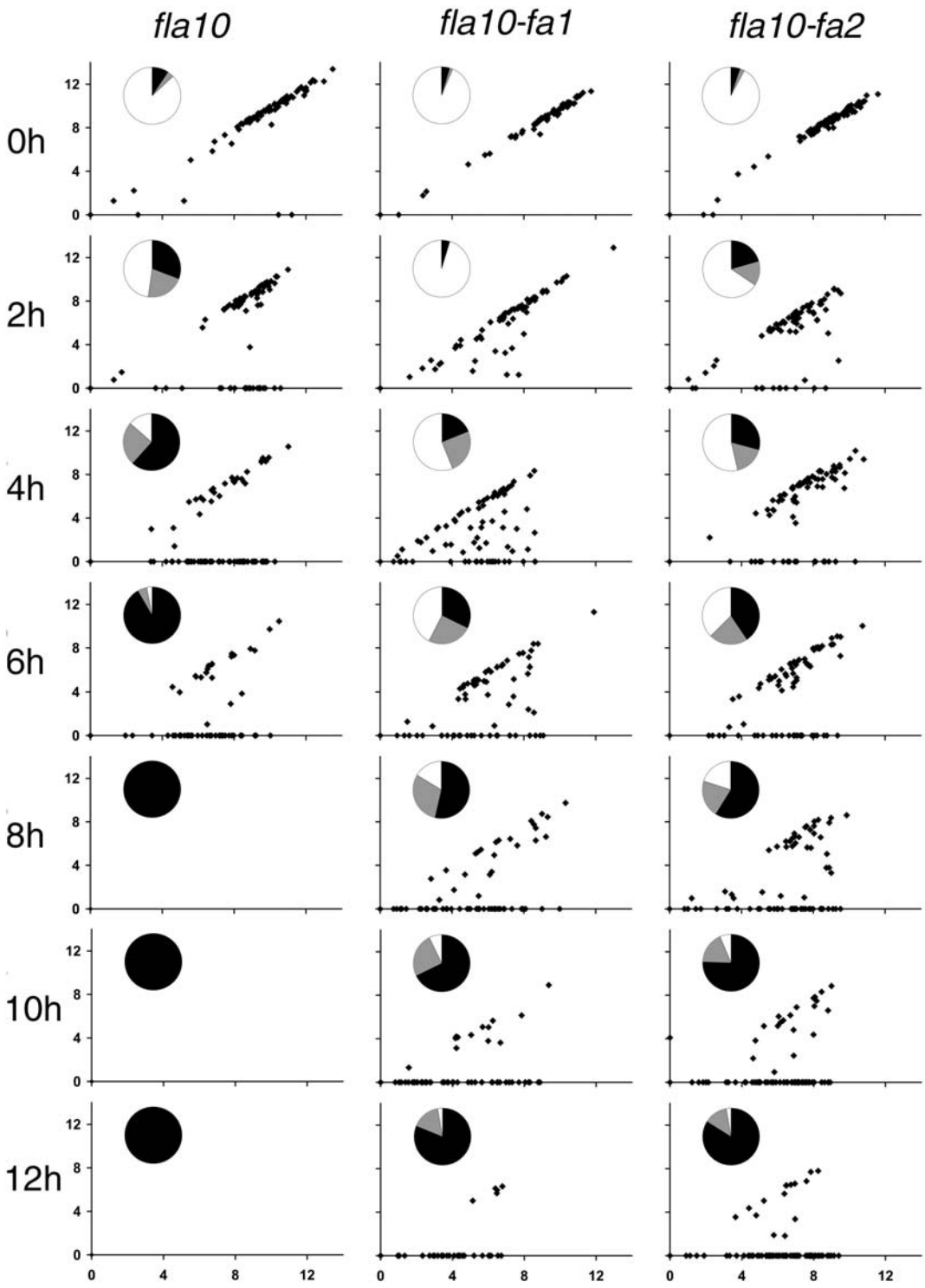

Figure 6
Double mutants of *fla10* with *fa1* or *fa2* are slower to disassemble flagella at the restrictive temperature than fla10 single mutants. Scatter plots of 33°C timecourses for *fla10*, *fla10-fa1* and *fla10-fa2*; plots prepared as described in the legend to Figure 3. TAP cultures were resuspended in pre-warmed TAP media at t = 0 h. No free flagella were seen in *fla10-fa1* or *fla10-fa2* cultures at any timepoint.

is not the case for *fla10* mutants at the restrictive temperature. In agreement with this, *fla2* cultures contain fewer uniflagellate cells than *fla10* cultures at the restrictive temperature, and this is likely due to the ability of *fla2*, but not *fla10*, to reassemble flagella at the restrictive temperature.

The observation of unequal shortening of flagella in a resorbing population (Figure 6) parallels the observation that one flagellum is prone to deflagellate before the other (Figures 3, 4, 6 and data not shown). Loss of one flagellum at a time is not observed in response to a strong deflagellation signal, but is observed after treatment with weaker stimuli such as a mild acid shock (data not shown) or threshold concentrations of alcian blue [33]. The two flagella of *Chlamydomonas* are not equivalent; the *cis* flagellum is defined as the one closest to the eyespot. Rather than the random loss of either flagellum, we hypothesize that the *cis* and *trans* flagella are differentially sensitive to disassembly signals, as they are to motility-related calcium signals [34]. This hypothesis predicts that disassembly will be initiated earlier and/or proceed more rapidly in one flagellum, which could explain the abundance of unequal length flagella observed in Figure 6.

Resorption is mediated at the transition zone

On the basis of a survey of the modes of flagellar loss in unicellular algae and fungi, Bloodgood divided flagellar loss into several categories [23]. Examples were cited of the same organism (or even the same cell) undergoing flagellar loss sometimes by resorption and sometimes by deflagellation [23]. Rather than supposing that organisms undergo flagellar loss by different mechanisms at different stages of their life cycles, we contend that the mechanism of flagellar loss is conserved, and that subtle variations in signaling leads to the appearance of either deflagellation or various forms of resorption. This conserved mechanism would involve severing of the axoneme at the base of the flagellum.

We now summarize specific experimental data suggesting that resorption is mediated at the transition zone, the site of deflagellation, rather than by constitutive disassembly at flagellar tips. First, *fla10* and *fla2* mutant cells disassemble their flagella at the restrictive temperature via deflagellation in the presence of calcium, and via resorption in the absence of added calcium. Second, *fa* mutants, which are unable to deflagellate, are slow to resorb; this effect would not be predicted if resorption were entirely due to dynamics at flagellar tips. The slow resorption phenotype of *fa1* is especially informative, as this mutant is slow to assemble flagella (JDKP, Ben Montpetit, LMQ, unpublished observations), in which case the length-control theory predicts that *fa1* should be fast to resorb. Third, the slow resorption of *fla10-fa* double mutant cells produces uniflagellate cells in the absence of deflagellation, a consequence not predicted by the dynamic length control model but in accordance with the results of mild deflagellation treatments. Fourth, while *Chlamydomonas* cells resorb their flagella prior to mitosis [24], EM studies have provided evidence that flagella are detached from basal bodies, but not lost from cells, during pre-mitotic resorption [29]. This seeming contradiction may be explained by our proposal that resorption requires microtubule severing activity at the flagellar transition zone.

There are also some theoretical considerations. Not only the tips of the outer doublet microtubules, but rather the whole axoneme, has been shown to undergo turnover in sea urchin embryos [35]. If this turnover occurs in all eukaryotic flagella, then flagellar tip dynamics alone [8] or lattice translocation models [36] could potentially account for this turnover. However, we believe a regulated ratcheting of the axoneme at the transition zone, coupled with dynamic activity at the tip, provides the most satisfying explanation for the occurrence of axonemal turnover even while a flagellum remains functional. Additionally, there is the consideration that flagellar length in *Chlamydomonas* is cell-cycle dependent, and flagella undergo a period of slow resorption prior to a period of fast resorption preceding cell division [8]. We hypothesize that the slow phase of resorption is mediated by the length-control mechanism, while the fast phase of resorption is mediated by severing of axonemal microtubules in the transition zone.

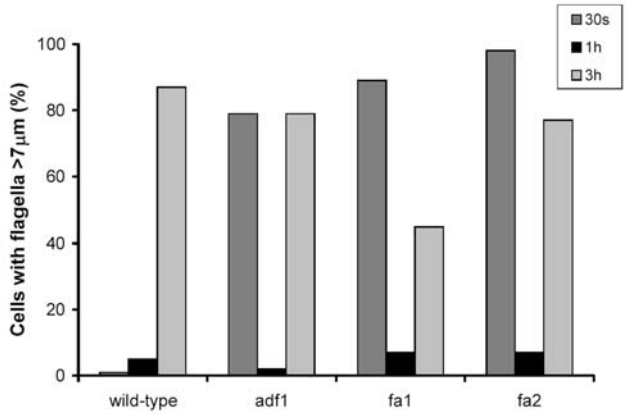

Figure 7
Acid shock causes resorption of mutants unable to deflagellate. At the indicated times post-acid shock, flagellar length distributions were determined and percents of cells with long flagella (flagella longer than 7 μm) were plotted.

The flagellar disassembly pathway

The deflagellation phenotype of *fla10* is significant, as it implies an active signalling event is at work. We predicted that if the deflagellation severing complex is responsible for resorption, then providing a deflagellation stimulus to a cell unable to deflagellate should induce resorption. Indeed, this is consistent with temperature-sensitive resorption of *fla10* in the absence of added calcium, and with temperature-induced resorption of *fla10-fa* double mutants with or without added calcium. To investigate whether this effect is more general, or only due to some special nature of the *fla* phenotype, we examined the effect of pH shock on mutants unable to respond by deflagellation. As previously shown, acid treatment of *fa1*, *fa2*, or *adf1* mutants does not lead to deflagellation as it does for wild-type cells [37,38]. However, as predicted by our model, these mutants do indeed resorb their flagella within one hour after a 30 s acid shock (Figure 7). This resorption is not necessarily complete, and is complicated by the tendency of the acid-treated *fa* cells to coil their flagella starting from the distal ends. These coiled flagella get smaller over time, and before disappearing entirely they become dense, stumpy structures. Sanders and Salisbury noted that the *Chlamydomonas* centrin mutant, *vfl-2*, will sometimes resorb rather than deflagellate in response to the deflagellation agent, dibucaine [39]. We have further extended these results by performing deflagellation experiments on wild-type cells in low-calcium buffer. Acid shock, dibucaine treatment, and 42°C heat shock all induce flagellar resorption of wild-type cells in low calcium buffer (data not shown).

We conclude that resorption and deflagellation both may result from a "flagellar disassembly" signal. If the fast phase of flagellar resorption results from disassembly mediated by the severing complex at the transition zone, as we have argued, then a new picture of deflagellation emerges: calcium-mediated hyperactivation of the severing complex following a disassembly signal leads to deflagellation. This model exposes our lack of understanding of the endogenous signaling pathways that lead to flagellar disassembly. Flagellar disassembly occurring prior to mitosis or meiosis in *Chlamydomonas* must occur via resorption, even in medium containing calcium, as cultures typically contain few free flagella. If agents such as intracellular acidification, dibucaine, and heat shock induce deflagellation by hyperactivation of an endogenous disassembly pathway by causing an increase in intracellular calcium, then why should similar treatments in low calcium cause resorption? Two nonexclusive possibilities present themselves: either the severing apparatus has progressively greater activity at higher calcium thresholds, and has partial activity sufficient to mediate resorption without full calcium activation; or there is a calcium-independent signaling pathway which is induced by treatments such as intracellular acidification.

A candidate for providing such a signal is the IFT pathway. Recent work has suggested that IFT has signaling functions [40], and it has been suggested that these signalling functions are perhaps more important for flagellar assembly than the physical transport of flagellar components [5]. Halting IFT in the presence of calcium induces deflagellation, as we have shown with the IFT mutant *fla10*, in which IFT halts quickly after shift to the restrictive temperature [41]. *fla10* and *fla2* are not the only IFT mutants which deflagellate; we have observed deflagellation at the restrictive temperature for several other *fla* mutants (data not shown). As most *fla* mutants have defects at some point in the IFT cycle at the permissive temperature [16], it is likely that IFT is disrupted in these mutants at the restrictive temperature. As first mentioned anecdotally by Kozminski et al. [41], halting IFT seems to lead to deflagellation.

As IFT is so intimately involved in flagellar assembly, it should not come as a surprise that it may play a role in flagellar disassembly. It is intuitive that a cell should not attempt to continue building a flagellum at a time when it is actively disassembling a flagellum, and this logic suggests that signalling events that regulate IFT should be related to flagellar disassembly signals. Deflagellation of *fla10* at the restrictive temperature, when anterograde IFT is halted, raises the possibility that functional IFT counteracts a constitutive disassembly signal. This could be adaptive. For example, for cells with damaged flagella, deflagellation would be the favored disassembly response, due to its speed and ability of *Chlamydomonas* to upregulate genes required for flagellar assembly post-deflagellation. If deflagellation is blocked, flagellar disassembly will instead occur via resorption.

Conclusions

We find that temperature-sensitive flagellar loss in the *Chlamydomonas* IFT mutants *fla10* and *fla2* is not due to constitutive resorption at flagellar tips, which plays a crucial role in flagellar length control, but rather due to activation of a disassembly pathway (Figure 8). We also find that flagellar disassembly can occur either by deflagellation or resorption in response to the same stimuli, and that deflagellation is preferred if sufficient calcium is available. Mutants unable to deflagellate are also slow to resorb flagella. We propose that resorption is actively mediated in the transition zone at the base of the flagellum, and that cellular signals which regulate IFT may also regulate flagellar disassembly.

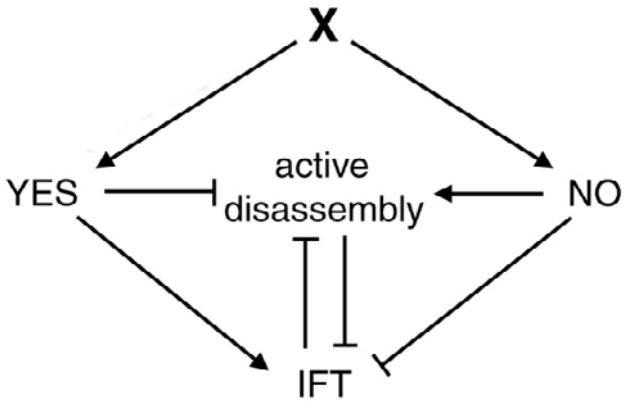

Figure 8
Flagella: to have or to have not. Model for the co-regulation of flagellar assembly (mediated by IFT) and active flagellar disassembly by a common signal, X. Under conditions either permissive or nonpermissive for flagellar assembly, IFT and disassembly are proposed to be mutually antagonistic. Under conditions appropriate for flagellar assembly (Yes), the decision is made to activate IFT and block disassembly at the transition zone. Active IFT could provide a signal to inhibit active disassembly. When flagella are to be disassembled (No), IFT is inactivated. In the absence of IFT, an inhibitory signal would be removed, allowing disassembly to proceed. There may also be direct activation of disassembly.

Methods

Strains

Chlamydomonas reinhardtii mutants *fla2* (CC-1390) and *fla10-1* (CC-1919) were provided by the *Chlamydomonas* Genetics Center. Wild-type strain B214 was provided by Dr. G. Pazour (University of Massachusetts). The *fa1-1* mutant was isolated by R.A. Lewin [42]; the *fa2-1* and *adf1-5* mutants were isolated in our lab [38]. Genetic crosses to obtain the double mutants *fla10-fa1-1*, *fla10-fa2-1*, *fla2-fa1-1*, and *fla2-fa2-1* were performed by standard techniques [30]. All cells were maintained on TAP (Tris-Acetate-Phosphate) media [30] under constant illumination at 20°C.

Since *Chlamydomonas* cells transiently resorb their flagella after resuspension in HEPES buffer (10 mM HEPES-KOH, pH 7.2) (with or without added calcium), cultures were incubated overnight in HEPES prior to HEPES experiments. No chelators were used in the preparation of low-calcium HEPES buffer as trace amounts of calcium are required for cell viability over the length of the experiments.

Timecourses and flagella length measurements

Cells were resuspended at 3 × 10^6 cells/mL in buffer or media, pre-warmed to the temperature of the incubation, at time zero. We find that the flagellar loss phenotypes of *fla10* are very temperature-sensitive, and a 1°C temperature difference can have a large effect on the kinetics of the experiment; this likely accounts for varying reports on the time required for *fla10* cultures to become bald. Thus, the same culture of cells was used for both +/- calcium at each temperature. Samples were fixed in 2% gluteraldehyde for flagellar length measurements and free flagella determinations. Cells were examined under DIC on an Olympus IX70 microscope (Carsen Group Inc., Ontario, Canada) and lengths of both flagella on at least 70 cells were measured using software provided with the DeltaVision system (Applied Precision, Seattle, Washington). All experiments were repeated on at least three independent cultures.

Authors' Contributions

JDKP carried out all experiments described in this work and drafted the manuscript. LMQ assisted in the design of the study, analysis of the data, and finalization of the manuscript.

Acknowledgements

We thank Doug Cole for directing our interest towards *fla2*; Arden Ma for initial analysis of the *fla2* deflagellation phenotype in our lab; Elizabeth Harris and the *Chlamydomonas* Genetics Center for strains; our colleagues at SFU for illuminating discussions; and an anonymous reviewer for several helpful remarks. JDKP thanks Rosemary Redfield for inspiration. This work was funded by the Natural Sciences and Engineering Research Council (NSERC, RGPIN 227132).

References

1. Kozminski KG, Johnson KA, Forscher P and Rosenbaum JL: **A motility in the eukaryotic flagellum unrelated to flagellar beating.** *Proc Natl Acad Sci USA* 1993, **90:**5519-5523.
2. Cole DG, Diener DR, Himelblau AL, Beech PL, Fuster JC and Rosenbaum JL: ***Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons.** *J Cell Biol* 1998, **141:**993-1008.
3. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB and Cole DG: ***Chlamydomonas* IFT88 and its mouse homologue polycystic kidney disease gene tg737 are required for assembly of cilia and flagella.** *J Cell Biol* 2000, **151:**709-718.
4. Rosenbaum JL and Witman GB: **Intraflagellar transport.** *Nat Rev Mol Cell Biol* 2002, **3:**813-825.
5. Cole DG: **The intraflagellar transport machinery of *Chlamydomonas reinhardtii*.** *Traffic* 2003, **4:**435-442.
6. Matsuura K, Lefebvre PA, Kamiya R and Hirono M: **Kinesis-II is not essential for mitosis and cell growth in *Chlamydomonas*.** *Cell Motil Cytoskel* 2002, **52:**195-201.
7. Pazour GJ, Wilkerson CG and Witman GB: **A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT).** *J Cell Biol* 1998, **141:**979-992.
8. Marshall WF and Rosenbaum JL: **Intraflagellar transport balances continuous turnover of outer doublet microtubules: implications for flagellar length control.** *J Cell Biol* 2001, **155:**405-414.
9. Marshall WF: **Size control in dynamic organelles.** *Trends Cell Biol* 2002, **12:**414-419.
10. Berman SA, Wilson NF, Haas NA and Lefebvre PA: **A novel MAP kinase regulates flagellar length in *Chlamydomonas*.** *Curr Biol* 2003, **13:**1145-1149.

11. Vashishtha M, Walther Z and Hall JL: **The kinesin-homologous protein encoded by the *Chlamydomonas* FLA10 gene is associated with basal bodies and centrioles.** *J Cell Biol* 1994, **126:**175-188.
12. Adams GMW, Huang B and Luck DJL: **Temperature-sensitive assembly-defective flagella mutants of *Chlamydomonas reinhardtii*.** *Genetics* 1982, **100:**579-586.
13. Vale RD: **The molecular motor toolbox for intracellular transport.** *Cell* 2003, **112:**467-480.
14. Huang B, Rifkin MR and Luck DJL: **Temperature-sensitive mutations affecting flagellar assembly and function in *Chlamydomonas reinhardtii*.** *J Cell Biol* 1977, **72:**67-85.
15. Piperno G, Siuda E, Henderson S, Segil M, Vaananen H and Sassaroli M: **Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects.** *J Cell Biol* 1998, **143:**1591-601.
16. Iomini C, Babaev-Khaimov V, Sassaroli M and Piperno G: **Protein particles in *Chlamydomonas* flagella undergo a transport cycle consisting of four phases.** *J Cell Biol* 2001, **153:**13-24.
17. Quarmby LM: **Cellular deflagellation.** *Int Rev Cytology* 2003.
18. Quarmby LM: **Ca^{2+} influx activated by low pH in *Chlamydomonas*.** *J Gen Physiol* 1996, **108:**351-361.
19. Lohret TA, McNally FJ and Quarmby LM: **A role for katanin-mediated axonemal severing during *Chlamydomonas* deflagellation.** *Mol Biol Cell* 1998, **9:**1195-1207.
20. Finst RJ, Kim PJ, Griffis ER and Quarmby LM: **Fa1p is a 171 kDa protein essential for axonemal microtubule severing in *Chlamydomonas*.** *J Cell Sci* 2000, **113:**1963-1971.
21. Mahjoub MR, Montpetit B, Zhao L, Finst RJ, Goh B, Kim AC and Quarmby LM: **The *FA2* gene of *Chlamydomonas* encodes a NIMA family kinase with roles in cell cycle progression and microtubule severing during deflagellation.** *J Cell Sci* 2002, **115:**1759-1768.
22. Lohret TA, Zhao L and Quarmby LM: **Cloning of the *Chlamydomonas* p60 katanin and localization to the site of outer doublet severing during deflagellation.** *Cell Motil Cytoskel* 1999, **43:**221-231.
23. Bloodgood RA: **Resorption of organelles containing microtubules.** *Cytobios* 1974, **9:**143-161.
24. Cavalier-Smith T: **Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardii*.** *J Cell Sci* 1974, **16:**529-556.
25. Rieder CL, Jensen CG and Jensen LCW: **The resorption of primary cilia during mitosis in a vertebrate $(PtK)_1$ cell line.** *J Ultrastr Res* 1979, **68:**173-185.
26. Tucker RW and Pardee AB: **Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells.** *Cell* 1979, **17:**527-535.
27. Pazour GJ and Witman GB: **The vertebrate primary cilium is a sensory organelle.** *Curr Opin Cell Biol* 2003, **15:**105-10.
28. Lewin RA and Lee KW: **Autotomy of algal flagella: electron microscope studies of Chlamydomonsa (Chlorophyceae) and Tetraselmis (Prasinophyceae).** *Phycologia* 1985, **24:**311-316.
29. Johnson UG and Porter KR: **Fine structure of cell division in *Chlamydomonas reinhardi* basal bodies and microtubules.** *J Cell Biol* 1968, **38:**403-425.
30. Harris EH: ***The* Chlamydomonas *Sourcebook*.** *Berkeley: Academic Press* 1989.
31. Lux FG 3rd and Dutcher SK: **Genetic interactions at the *FLA10* locus: suppressors and synthetic phenotypes that affect the cell cycle and flagellar function in *Chlamydomonas reinhardtii*.** *Genetics* 1991, **128:**549-561.
32. Rosenbaum JL, Moulder JE and Ringo DL: **Flagellar elongation and shortening in *Chlamydomonas*.** *J Cell Biol* 1969, **41:**600-619.
33. Evans JH and Keller LR: **Receptor-mediated calcium influx in *Chlamydomonas reinhardtii*.** *J Euk Microbiol* 1997, **44:**237-245.
34. Kamiya R and Witman GB: **Submicromolar levels of calcium control the balance of beating between the two flagella in demembranated models of *Chlamydomonas*.** *J Cell Biol* **98:**97-107.
35. Stephens RE: **Synthesis and turnover of embryonic sea urchin ciliary proteins during selective inhibition of tubulin synthesis and assembly.** *Mol Biol Cell* 1997, **8:**2187-2198.
36. Stephens RE: **Preferential incorporation of tubulin into the junctional region of ciliary outer doublet microtubules: a model for treadmilling by lattice dislocation.** *Cell Motil Cytoskel* 2000, **47:**130-140.
37. Quarmby LM and Hartzell HC: **Two distinct calcium-mediated signal transduction pathways can trigger deflagellation in *Chlamydomonas reinhardtii*.** *J Cell Biol* 1994, **124:**807-15.
38. Finst RJ, Kim PJ and Quarmby LM: **Genetics of the deflagellation pathway in *Chlamydomonas*.** *Genetics* 1998, **149:**927-936.
39. Sanders MA and Salisbury JL: **Centrin plays an essential role in microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*.** *J Cell Biol* 1994, **124:**795-805.
40. Pan J and Snell WJ: **Kinesin-II is required for flagellar sensory transduction during fertilization in Chlamydomonas.** *Mol Biol Cell* 2002, **13:**1417-26.
41. Kozminski KG, Beech PL and Rosenbaum JL: **The *Chlamydomonas* kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane.** *J Cell Biol* 1995, **131:**1517-1527.
42. Lewin RA and Burrascano C: **Another new kind of *Chlamydomonas* mutant, with impaired flagellar autotomy.** *Experimentia* 1983, **39:**1397-1398.