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Synthesis and Assembly of Flagellar Components by *Caulobacter crescentus* Motility Mutants

REID C. JOHNSON,† DONNA M. FERBER,‡ AND BERT ELY*

Department of Biology, University of South Carolina, Columbia, South Carolina 29208

Received 29 November 1982/Accepted 16 March 1983

Cultures of wild-type *Caulobacter crescentus* and strains with *fla* mutations representing 24 genes were pulse-labeled with ^{14}C-amino acids and analyzed by immunoprecipitation to study the synthesis of flagellar components. Most *fla* mutants synthesize flagellin proteins at a reduced rate, suggesting the existance of some mechanism to prevent the accumulation of unpolymerized flagellin subunits. Two strains contain deletions that appear to remove a region necessary for this regulation. The hook protein does not seem to be subject to this type of regulation and, in addition, appears to be synthesized as a faster-sedimenting precursor. Mutations in a number of genes result in the appearance of degradation products of either the flagellin or the hook proteins. Mutations in *flaA*, *-X*, *-Y*, or *-Z* result in the production of filaments (stubs) that contain altered ratios of the flagellin proteins. In some *flaA* mutants, other flagellin-related proteins were assembled into the stub structures in addition to the flagellins normally present. Taken together, these analyses have begun to provide insight into the roles of individual *fla* genes in flagellum biogenesis in *C. crescentus*.

Caulobacter crescentus undergoes a unique developmental cycle in which the cell spends approximately one-third of its life as a motile swarmer cell and the remainder as a nonmotile stalked cell (18, 19). The swarmer cell is essentially an immature cell that is incapable of DNA replication. It is characterized by a single polar flagellum that is composed of a filament, a hook, a rod, and five basal rings (8). As the swarmer cell matures, the flagellum, minus the basal rings, is released into the culture medium, (8, 20), and the cell synthesizes a stalk at the formerly flagellated pole. The new stalked cell begins DNA replication, elongates, and finally synthesizes a new flagellum at the pole opposite the stalk. The resulting predivisional cell undergoes an asymmetric cell division yielding a new swarmer cell and regenerating the stalked cell (18, 19).

The flagellar filament has been found to be composed of two proteins, a minor species with a subunit molecular weight of 27,500 (27K) and the major species with a subunit molecular weight of 25K (3, 10, 13, 21). Purified hooks are comprised of a protein with a subunit molecular weight of approximately 70K (8, 12, 22). The synthesis of both flagellins and of the hook protein have been followed in synchronized cell populations by radioimmunoassay, and both are synthesized coincident with their assembly (11, 15, 23). Furthermore, by using DNA synthesis inhibitors and mutants temperature sensitive in DNA elongation, it has been found that the synthesis of both of these flagellar component appears to be coupled with the completion of DNA synthesis, although not neccessarily in the same step (15, 16).

Recently, we have isolated approximately 70 nonmotile mutants of *C. crescentus* and have identified over 30 genes that are involved in flagellum synthesis, assembly, and rotation (7). These include 24 *fla*, 3 *mot*, and 3 *ple* genes (7; Johnson and Ely, unpublished data). In this study, we have analyzed the intracellular and assembled flagellins in the wild type and in the *fla* mutants to further our understanding of the mechanism of temporal control of flagellin biosynthesis, the process of assembly of the filaments, and the function of the various genes we have identified genetically.

MATERIALS AND METHODS

Bacterial strains and media. The *C. crescentus* wild-type strain CB15 and the spontaneous nonmotile mutants derived from it, along with their gene assignments, have been described by Johnson and Ely (7). Complete (PYE) and minimal (M2) media have been described previously (6).

Immunoprecipitation assay for intracellular flagellin and hook proteins. Total intracellular flagellin and hook protein was assayed by using a procedure similar to the radioimmune assay described by Lagenaur and

† Present address: Department of Biochemistry, University of Wisconsin, Madison, WI 53706.
‡ Present address: Synergen, Boulder, CO 80301.

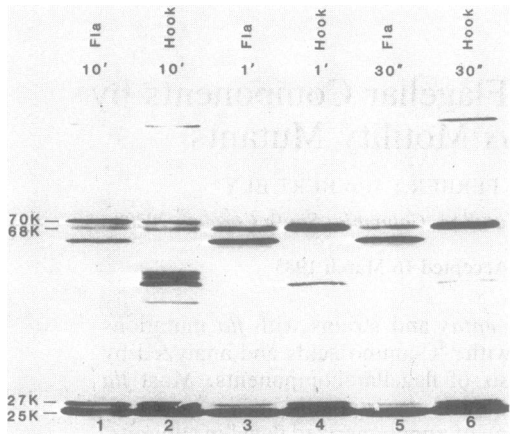

FIG. 1. Immunoprecipitation of pulse-labeled proteins with antiflagellum and antihook antibody. Cultures were pulse-labeled with a mixture of ^{14}C-amino acids for the times indicated (10', 10 min; 30", 30 s), lysed, and immunoprecipitated with either antiflagellum (Fla) or antihook (Hook) antibody. The positions of the flagellin and hook proteins are indicated on the left.

Agabian (11). Cells were grown at 33°C in M2 medium supplemented with 0.3% glucose to 100 Klett units (no. 54 green filter). Typically, 5 μCi of ^{14}C-amino acids (yeast profile reconstituted protein hydrolysate; Amersham Corp.) was added to 2 ml of culture, and incubation was continued for 10 min. The cells were chilled, collected by centrifugation, washed once with 10 mM Tris (pH 7.5), and suspended in 0.5 ml of 10 mM Tris–10 mM EDTA (pH 7.5). Lysozyme (Sigma Chemical Co.) was added to a final concentration of 200 μg/ml, and the suspension was incubated for 30 min at 33°C. Triton X-100 (Sigma) was added to a final concentration of 1%, and the samples were vortexed vigorously. The lysates were then sheared repeatedly through a 26-gauge needle and centrifuged at 30,000 × *g* for 30 min. The supernatant was collected, and NaCl was added to a final concentration of 0.15 M. Fifteen microliters of purified immunoglobulin G (IgG) from anti-*C. crescentus* flagella or anti-*C. crescentus* flagellin rabbit serum (gifts from A. Weissborn and L. Shapiro) was added along with 3 μg of purified acid-dissociated flagellin. Alternatively, 100 μl of purified IgG from anti-*C. crescentus* hook goat serum was added along with 3 μg of acid-dissociated hook protein. The reaction mixture was incubated at 37°C for 1 h and then overnight at 4°C.

The precipitates were collected and purified by layering the reaction mixture over 0.5 ml of 1 M sucrose in phosphate-buffered saline (10 mM sodium phosphate, pH 7.5, plus 0.15 M NaCl) containing 10 mM EDTA and 1% Triton X-100 in 1.5-ml microfuge tubes. After centrifugation at 10,000 × *g* for 10 min, the top layer was removed and replaced with phosphate-buffered saline–EDTA–Triton X-100, and the contents of the tube were aspirated to the bottom, leaving the immunoprecipitate in the pellet. The precipitate was dissolved by vortexing vigorously and heating to 100°C for 2 min in 50 μl of Laemmli sample buffer (9). The proteins were separated by electrophoresis on a 7.5 to 20% gradients sodium dodecyl sulfate (SDS)-polyacrylamide slab gel (1) and examined by autoradiography. In some cases, the gel was impregnated with En^{3}Hance (New England Nuclear Corp.) or with a solution containing 2 g of 2-methylnaphthalene and 0.4 g of 2,5-diphenyloxazole in 100 ml of glacial acetic acid with gentle shaking for 1 h (17). This treatment was followed by precipitation with water for 1 h before exposure to X-ray film (Kodak XAR) for 4 to 7 days at −70°C.

Isolation of defective filaments (stubs). Defective filaments were isolated and purified from the growth media by the protocol for the purification of hook structures lacking flagellin filaments described previously (8), except that Triton X-100 and EDTA were added to final concentrations of 1% and 10 mM, respectively, immediately before the first ultracentrifugation.

RESULTS

Wild-type flagellin and hook proteins. Purified *C. crescentus* flagella are comprised of two flagellin proteins with subunit molecular weights of 25K and 27K, a hook protein with a subunit molecular weight of 70K, and trace amounts of a protein that is likely to be the rod protein and has a subunit molecular weight of 32K (8). To examine the synthesis of these proteins, we used antibodies raised against either released flagella containing the hook and rod structures or hook and rod structures lacking the filament and purified from mutant cell culture fluids (8) to precipitate proteins from extracts of cultures pulse-labeled with ^{14}C-amino acids. Antibody prepared against released flagella precipitated the 25K, 27K, and 70K proteins (Fig. 1, lane 1). Presumably, ^{14}C-labeled 32K protein was not present in sufficient amounts in the cell extracts to be detected in these experiments. In addition to these proteins, several other bands were detected. The protein with a subunit molecular weight of 68K appeared to be related to the 70K hook protein (see below). The remaining protein band observed in Fig. 1, lane 1, can be precipitated by some preimmune rabbit sera (25). The same intensity of labeling of flagellin and hook protein bands was obtained when the antiflagellin antibody precipitate was coprecipitated with goat anti-rabbit IgG serum in the absence of unlabeled carrier flagellin, indicating that our carrier-mediated assay quantitatively recovers flagellin and hook proteins (data not shown). Additional bands could be observed in the autoradiograms, but are unlikely to be related to flagella since they are unaffected by *fla* mutations (see below) and are not precipitated by both antiflagellum and antihook antibody. In some experiments the pattern of high-molecular-weight bands appeared similar to the surface array proteins observed by Smit et al. (25) and

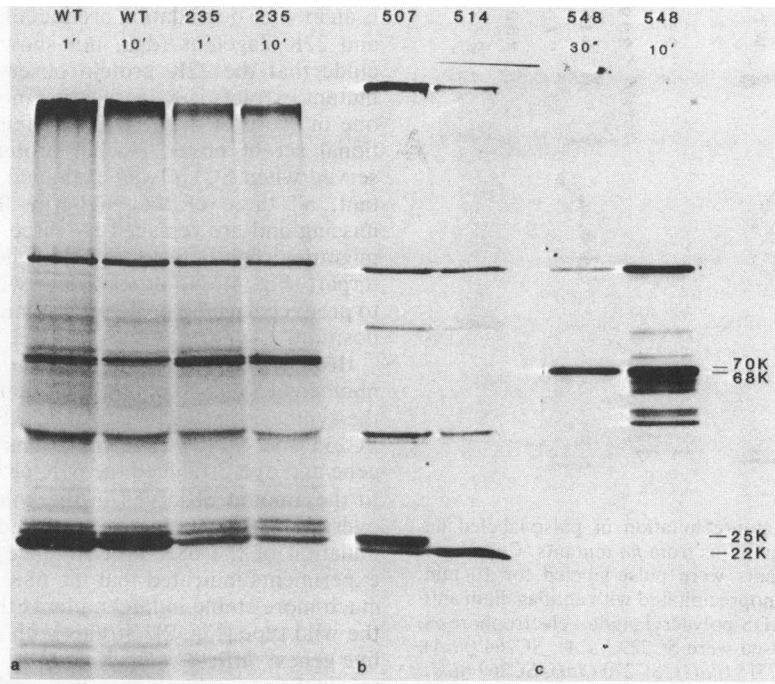

FIG. 2. Immunoprecipitation of flagellin and hook proteins after brief labeling periods. (a) Cultures of the wild type and SC235 (*flaQ*) were pulse-labeled for 1 min, filtered through a 0.45-μm filter, suspended in fresh medium, and either put on ice (1 min, 1') or incubated for 10 min at 33°C (10'). Cells were then lysed and immunoprecipitated with antiflagellum antibody. (b) Cultures of SC507 (with deletion of *flaEF*) and SC514 (with deletion of *flaEF*) were pulse-labeled for 10 min, lysed and immunoprecipitated with antiflagellum antibody. (c) Cultures of SC548 (*flaP*) were pulse-labeled for 30 s (30") or 10 min, lysed, and immunoprecipated with antihook antibody.

could be due to copurification of small amounts of the array with flagella.

When cells were pulse-labeled for 1 min or 30 s, the 68K protein was labeled intensely, and the 70K protein was barely detectable (Fig. 1, lanes 3 and 5). After 10 min, the intensity of the 68K protein band was reduced, whereas the intensity of the 70K hook protein band was significantly increased (Fig. 1, lane 1). Furthermore, after 10 min of growth in the absence of label, the 68K protein band had nearly disappeared, indicating that it is only transiently present (Fig. 2). These results suggested that the 68K protein could be a precursor to one of the other proteins, perhaps the 70K protein. To test this hypothesis, we used antibody raised against purified hook structures to precipitate proteins from extracts of cells pulse-labeled for 10 min, 1 min, or 30 s (Fig. 1, lanes 2, 4, and 6). Antihook antibody would be expected to precipitate flagellin monomers that have been assembled onto hook structures, and little difference was observed with the 25K and 27K flagellin bands. When the 68K and 70K proteins were examined, the 68K protein band was again more heavily labeled, and the 70K protein was barely detectable with short labeling periods (Fig. 1, lanes 4 and 6). Since none of the other bands was precipitated by both antibodies, these results are consistent with the idea that the 68K protein is a precursor of the 70K protein. The 68K protein is not a flagellin protein since experiments with antibody raised against purified flagellin did not result in the precipitation of a 68K band (data not shown).

Flagellin proteins produced by mutants lacking a normal flagellum (*fla*). Johnson and Ely (7) divided 61 *fla* mutants into 26 linkage groups based on transductional mapping. Recent experiments with more refined genetic mapping techniques suggested that *flaG* and *flaT* are in the same linkage group and that *flaP* and *flaU* are in the same linkage group (Ely et al., manuscript in preparation). Representative nonmotile mutants defective in each of the 24 *fla* genes currently identified were assayed for the presence of flagellin and hook proteins by immunoprecipitation with antiflagellum antibody. Every mutant examined, except SC507 and SC514, had reduced levels of the 25K and 27K proteins, suggesting that flagellin synthesis was reduced in strains incapable of assembling a flagellum. SC507 and SC514 contain extended deletions in

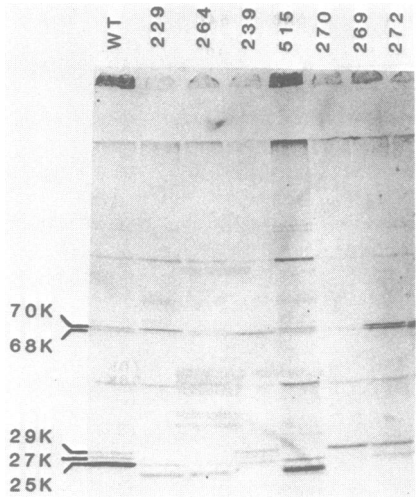

FIG. 3. Immunoprecipitation of pulse-labeled flagellin and hook proteins from *fla* mutants. Cultures of various *fla* mutants were pulse-labeled for 10 min, lysed, and immunoprecipitated with antiflagellum antibody before SDS-polyacrylamide electrophoresis. Mutant strains used were SC229 (*flaA*), SC264 (*flaA*), SC239 (*flaC*), SC515 (*flaG*), SC270 (*flaI*), SC269 (*flaJ*), and SC272 (*flaN*).

the *flaEF* region (7) and synthesized the flagellin-related proteins at a rate equal to or greater than that observed with the wild type (Fig. 2). Also, it can be observed in Fig. 3 and 4 that a third flagellin-related protein with a subunit molecular weight of 29K was present in the wild-type strain and also in many of the mutants. This protein is not detectable in the wild-type flagellar filament and is not precipitated by all antiflagellum antibody preparations (Fig. 1). However, it appears to be a flagellin protein, since (i) it is synthesized at the same time in the cell cycle as the other flagellum proteins (11), (ii) its synthesis was altered by *flaA*, *-G*, or *-R* mutations (Fig. 3 and 4), (iii) strains containing certain *flaA* mutations assembled a defective flagellar structure containing the 29K protein (see below) and (iv) the 29K protein was not precipitated by antihook antibody (except when it was assembled into defective flagellar structures).

A mutation in any one of three genes, *flaA*, *-G*, and *-R*, resulted in the production of a novel protein with a subunit molecular weight of 22K and was usually correlated with the loss of the 29K and 27K flagellins and a reduction of the 25K flagellin (Fig. 3 and 4). When the labeling period was reduced to 30 s for these mutants, the amount of radioactivity in the 22K protein was reduced severely, and at longer labeling periods it increased in parallel with the intensity of labeling of the 25K protein (data not shown). Since we also have observed that a 22K protein is an in vitro degradation product of purified 25K and 27K flagellins (data not shown), we conclude that the 22K protein observed in these mutant extracts is a degradative intermediate of one or more of the flagellin proteins. An additional set of novel flagellin proteins was observed when SC175 was examined. In this mutant, all three of the wild-type flagellins are missing and are replaced by three bands, each migrating slightly faster than its wild-type counterpart (Fig. 4). No other strains were observed to produce flagellin proteins that migrated at this position.

Hook proteins produced by *fla* mutants. A number of strains contain mutations that affect the synthesis of the hook proteins. For instance, SC235 and SC307 have mutations in the *flaO* gene and overproduced the 68K protein relative to the amount observed in the wild type. This overproduction could have resulted from accumulation of the 68K protein since pulse-chase experiments indicated that the 68K protein was much more stable in *flaQ* mutants than it was in the wild type (Fig. 2). Strains with mutations in five genes, *flaB*, *-C*, *-D*, *-P*, and *-V*, were unable to synthesize a hook structure and in addition produced a set of novel proteins with subunit molecular weights of 64K and 66K. All of these proteins were precipitated by either antiflagellum or antihook antibody (Fig. 2 and 4, Table 1). The 64K protein was often seen in wild-type extracts at barely detectable levels, but the 66K protein has never been observed. When SC548 containing the *flaP165* mutation was examined after a 30-s labeling period, the 64K and 66K proteins were not observed even though the 68K protein was labeled intensely (Fig. 2). Thus, the 64K and 66K proteins are not synthesized as

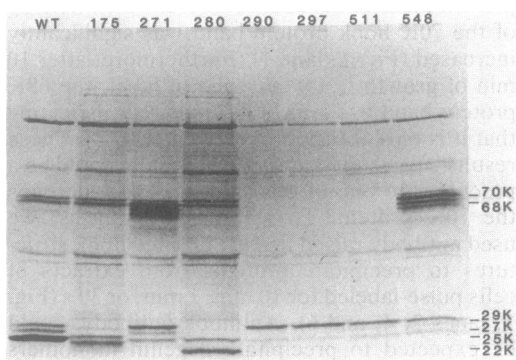

FIG. 4. Immunoprecipitation of pulse-labeled flagellin and hook proteins from *fla* mutants. Cultures of the wild type, SC175 (*flaZ*), SC271 (*flaD*), SC513 (*flaD*), and SC516 (*flaB*) were pulse-labeled for 10 min, lysed, and immunoprecipitated with antiflagellum antibody.

TABLE 1. Flaggelin and hook protein synthesis and assembly in *C. Crescentus fla* mutants

Mutant gene(s)	Assembled flagellar structures	Intracellular flagellins	Intracellular hook proteins	Possible defect
Wild type	Flagella with 27K and 25K flagellins and hook with 70K protein	29K, 27K, 25K	70K, 68K	None
flaA	Stubs with 29K, 27K, 25K (often doublet), and 22K proteins and hook with 70K protein	29, 27K (trace or absent), 25K, 22K	70K, 68K	Filament assembly
flaX, *-Y*	Stubs with 27K, 25K (*flaX* has doublet), and 70K protein	29K, 27K, 25K	70K, 68K	Filament assembly
flaG, *-R*	Hook with 70K protein	25K, 22K	70K, 68K	Filament assembly
flaE, *-F*, *-H*, *-N*	Hook with 70K protein	29K, 27K, 25K	70K, 68K	Filament assembly
flaZ	Stubs with 70K protein	28K, 26K, 24K	70K, 68K	Flagellin modification
flaJ	Large hook with 70K protein	29K, 27K, 25K	70K, 68K	Hook assembly
flaQ	Hook with 70K protein	29K, 27K, 25K	70K, 68K (overproduces)	Hook protein modification
flaB, *-C*, *-D*, *-P*, *-V*	None	29K, 27K, 25K	70K (trace), 68K, 66K, 64K	Hook protein modification
flaK, *-O*, *-W*, *-S*	None	29K, 27K, 25K	None	Hook protein synthesis
flaL, *-M*	None	29K, 27K, 25K	70K (trace)	Hook protein synthesis
flaI	None	29K	68K	Unknown

rapidly as the 68K or flagellin proteins. The 70K protein is barely detectable in these strains.

Mutations in several other genes affected the two hook proteins. For instance mutations in *flaC*, *-I*, and *-M* result in the production of trace amounts of the 70K protein, and *flaI* and *-M* mutants result in reduced amounts of the 68K protein as well (Fig. 3). Strains containing mutations in *flaK*, *-O*, *-S*, or *-W* do not produce either of the two hook proteins (data not shown). Therefore, it is possible that one of these four genes is the structural gene for the hook protein.

Defective structures produced by *fla* mutants. Johnson et al. (8) showed that many mutants lacking an intact flagellum synthesized an apparently normal hook structure. These hook structures were released into the culture medium and could be isolated and examined either by electron microscopy or by SDS- polyacrylamide gel electrophoresis. We have used both methods to examine culture fluids from mutants representative of each linkage group. Hook structures were not present in culture fluids from strains containing mutations in *flaB*, *-C*, *-D*, *-I*, *-K*, *-L*, *-M*, *-O*, *-P*, *-S*, *-V*, and *-W*. In contrast, culture fluids from strains with mutations in *flaE*, *-F*, *-G*, *-H*, *-N*, *-Q*, *-R*, *-Z*, and some with *flaA* mutations contained structures that appeared to be normal except for the lack of the flagellar filament. Other strains produced aberrant structures. For instance, we had shown previously that SC269 produced hook structures that contained normal proteins, but had an abnormal morphology (8). The remaining strains, SC255 (*flaX*), SC288 (*flaX*), SC274 (*flaY*), SC175 (*flaZ*), SC176 (*flaA*), SC230 (*flaA*), SC260 (*flaA*), and SC285 (*flaA*), produced apparently normal hook structures, but contained only small pieces of the flagellar filament (stubs). Stubs from these mutants were purified and examined by SDS-polyacrylamide gel electrophoresis to determine their protein content. Preparations obtained from the *flaA* mutants contained small amounts of 29K and 22K proteins in addition to the 25K and 27K proteins (Fig. 5). In some instances, the 25K protein could be resolved into a doublet (Fig. 5; SC230, 33°C). This phenomenon was never observed with wild-type flagellum preparations even when very small amounts were loaded on the gel. The observation of the 29K and 22K proteins in assembled flagellar structures provides additional evidence that these proteins are related to the 25K and 27K proteins.

In addition to the presence of the novel proteins, the stubs often contained altered ratios of the 25K and 27K proteins. Normally, the wild-type flagellum contains the 25K and the 27K flagellins in a ratio of 4:1 (10, 21). However, stubs from the *flaA* strains generally contained nearly equal quantities of the two flagellins, and

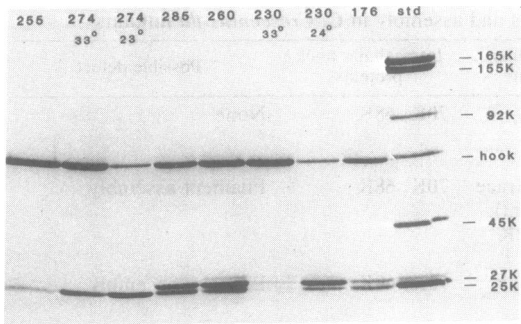

FIG. 5. SDS-polyacrylamide gel electrophoresis of purified stubs. Stubs released from cultures of the indicated *fla* mutants were purified and subjected to SDS-polyacrylamide gel electrophoresis. The mutants SC230 (*flaA*) and SC274 (*flaY*) are temperature sensitive for motility and were grown both at 33°C and at room temperature. SC255 (*flaX*), SC285 (*flaA*), SC260 (*flaA*), and SC176 (*flaA*) were grown at 33°C. The lane on the far right contained *C. crescentus* RNA polymerase, hooks, and flagella as molecular weight standards (1, 8).

those from the *flaX* and *flaY* mutants contained little or no 27K flagellin (Fig. 5). The significance of thes alterations is not clear since whole flagella isolated from the temperature-sensitive motility mutants SC174 (*flaY*) or SC230 (*flaA*) at a temperature permissive for motility also have altered ratios of the 25K and 27K proteins relative to that observed with wild-type flagella (Fig. 5). The step responsible for these altered ratios appears to be at the level of assembly, since the levels of the intracellular pools of the flagellins show no correlation with the assembled ratios (data not shown).

DISCUSSION

Johnson and Ely (7) found that a mutation in any one of a large number of genes could affect flagellum morphogenesis in *C. crescentus*. We have examined each of these mutants for the presence of assembled structures and for the ability to synthesize the flagellin and hook proteins. The results of these experiments are summarized in Table 1. Immunoprecipitation experiments of pulse-labeled cells showed that in addition to the 27K and 25K flagellins, 29K and 22K proteins were synthesized. Similarly, 68K, 66K, and 64K proteins were synthesized in addition to the 70K hook protein. The 22K and 29K proteins appear to be flagellins or derived from flagellins, since (i) they were precipitated by antiflagellum antibody, but not by antihook antibody, (ii) production of the 22K and 29K proteins was affected by mutations that result in the absence of a flagellum (iii) stubs were produced by some *flaA* mutants that contain the 22K and 29K proteins and (iv) the 22K and 29K proteins were synthesized with the same periodicity as the 25K and 27K flagellins (11). The results from short pulse-labeling periods indicated that the 29K protein is not a precursor to either the 27K or the 25K flagellins since all three proteins are observed after a 30-s labeling period as well as after a 1-min labeling period with a 10-min chase with unlabeled amino acids (Fig. 2). These results, along with the data showing that the 25K and the 27K flagellins are encoded by two distinct genes (4, 27), suggest that there may be three genes for the three flagellins. This conclusion is consistent with the observation of Milhausen et al. (14) that cDNA made from flagellin mRNA hybridizes to three discrete regions of the *C. crescentus* chromosome. In contrast to the 29K flagellin, the 22K protein appears to be a breakdown product of the 25K and 27K flagellins since it appears during storage of partially purified preparations of whole flagella and does not appear to be synthesized before the other flagellins. A similar suggestion has been made by Lagenaur and Agabian (11), and a 22K protein has been observed in immunoprecipitation experiments with *ple* mutants (2).

The 64K, 66K, and 68K proteins appear to be related to the 70K hook protein, since they are precipitated by antihook and antiflagellum antibodies. Although these three proteins could be fortuitously precipitated by the two antibodies, it is more likely that they are related to the hook protein since their synthesis is affected by mutations that affect the production of the hook structure. Pulse-labeling experiments for short periods indicate that the 68K protein is synthesized before the other hook-related proteins and turns over rapidly. The 64K, 66K, and 70K proteins all appear to be relatively stable. These results suggest that the 68K protein might be a precursor to the 64K, 66K, and 70K proteins. Since the 64K and 66K proteins are found in significant quantities only in certain *fla* mutants that have greatly reduced quantities of the 70K hook protein, it is possible that they are degradation products of one or both of the other two hook proteins.

One other mutant, SC175, produced altered proteins. In this case, all three flagellin proteins appeared to be slightly smaller on SDS-polyacrylamide gels. Since the three flagellins are encoded by at least two separate genes (4, 27), the size difference could not be due to a single in phase deletion. Therefore, we conclude that the SC175 mutant provides evidence for some sort of processing of the flagellin proteins.

One feature that was common to almost all of the *fla* mutants was the reduced synthesis of the 25K and 27K flagellin proteins. For example, in

strain SC269, where the presence of the *flaJ* mutation appears to result in a defect in the assembly of the hook structure (8), a reduced rate of synthesis was observed. This phenomenon was not observed with *che* or *mot* mutants where flagellar filaments were assembled. Therefore, we propose that in the absence of the assembly of a flagellar filament, the intracellular accumulation of flagellin subunits somehow reduces the rate of flagellin synthesis. The exceptional mutants, SC507 and SC514, produce flagellin proteins at a rate that appears to be the same as or faster than that of the wild type. Since these two mutants are deletions that do not recombine with the *flaE* or *flaF* genes, it is likely that the deletion removes a region that is responsible for the reduction of flagellin synthesis. Synthesis of the hook protein does not appear to be subject to this type of regulation since many mutants with reduced rates of flagellin synthesis as well as SC507 and SC514 have normal rates of hook synthesis. Furthermore, the inability to assemble a hook structure does not necessarily lead to a reduced rate of synthesis of the hook protein since large amounts of the degraded hook proteins are synthesized by *flaB*, *-C*, *-D*, *- P*, and *-V* mutants. Phenomena similar to that described above have been observed with *Escherichia coli* and *Salmonella typhimurium* (24, 26). In both of these organisms, no detectable flagellin is observed in *fla* mutants, and Suzuki and Iino (26) have shown that there is no detectable flagellin mRNA as well. In contrast, the hook structures are assembled normally in the absence of the flagellin protein (5). Thus, regulation of flagellin and hook protein synthesis appears to be similar in all three organisms, although the reduction of flagellin synthesis in *C. crescentus fla* mutants is less extreme than that observed in the other two bacteria.

Recently, it has been shown that the 27K flagellin is assembled preferentially at the proximal end of the flagellum produced by wild-type cells (3, 11, 27). Similarly, the distal portion of the flagellum is comprised primarily of the 25K flagellin. The reason for this spatial arrangement is not understood, although the 27K flagellin may be required for the efficient assembly of the filament. As discussed by Weissborn et al. (27), the altered ratios of the flagellin proteins found in the stub mutants and in the intact flagella of temperature-sensitive mutants may provide some clues toward understanding the spatial arrangement and assembly of the flagellar filament.

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