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Caulobacter crescentus Fatty Acid-Dependent Cell Cycle Mutant

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A fatty acid auxotroph of *Caulobacter crescentus*, AE6001, which displays a strict requirement for unsaturated fatty acids to grow on glucose as the carbon source has been isolated. Starvation of AE6001 for unsaturated fatty acids resulted in a block in the cell cycle. Starved cultures accumulated at the predivisional cell stage after a round of DNA replication had been completed and after a flagellum had been assembled at the pole of the cell. Cell division and cell growth failed to occur probably because the mutant was unable to synthesize a membrane. An analysis of double mutants containing the *fatB503* allele and other mutations in membrane biogenesis demonstrated that the cell cycle of AE6001 blocked at a homeostatic state. The addition of oleic acid to starved cultures permitted cell division and the initiation of a new round of DNA replication. The coincident block in both the initiation of DNA replication and membrane assembly, exhibited by starved cultures of this mutant, suggests that the *fatB503* gene product may be involved in the coordination of these events.

The timed expression of several sets of genes required for the differentiation of swarmer and stalked cells during the *Caulobacter* cell cycle appears to be dependent on both membrane lipid synthesis and DNA replication (3, 10, 13, 14). The synthesis of flagellin and hook protein components of the flagellum and a group of outer membrane proteins (3, 13) was found to be dependent on ongoing phospholipid synthesis. These same flagellar components (10, 14) and a subset of those membrane components that are dependent on lipid synthesis (13) are, in turn, dependent on the replication of the chromosome for their synthesis. Osley et al. (10) and Sheffery and Newton (14) have proposed that the replication of the chromosome (once during each division cycle) may act to signal the timed expression of a set of genes involved in the differentiation of the *Caulobacter* cell surface. A link between membrane lipid synthesis and DNA replication was established in *Caulobacter crescentus* when it was observed that either mutants in lipid synthesis or treatment with drugs which inhibit lipid synthesis in some way blocks the replication of the chromosome. To understand the manner in which DNA replication and membrane synthesis are coordinated and to determine the role of this dependent interaction in surface localization of differentially expressed proteins, we have been isolating lipid auxotrophs and analyzing their effect on the cell cycle (5, 13; D. Hodgson, P. Shaw, V. Letts, S. Henry, and L. Shapiro, submitted for publication).

We report here that a mutant auxotrophic for unsaturated fatty acids, AE6001, profoundly alters the cell cycle when it is starved for fatty acids. The earliest effect of fatty acid starvation that we observed was the cessation of fatty acid synthesis. This was followed by a block in phospholipid synthesis. DNA synthesis then came to a halt after an amount of time elapsed that was sufficient to complete a round of replication. In these cultures the cell cycle was found to block at the predivisional cell stage, upon completion of DNA replication and before cell division. Concomitant with the inhibition of DNA replication in AE6001, flagellar protein synthesis ceased, and three outer membrane proteins could not be detected in the cell membrane. The synthesis of both sets of proteins was previously shown to be dependent on DNA replication (10, 13).

Double mutants carrying *fatB503* and other membrane biogenesis mutations were constructed, and epistatic effects were analyzed. Based on these results and the observed cell cycle block of AE6001 upon starvation for fatty acids, we conclude that the genetic lesion in AE6001 is in a step that coordinates membrane lipid synthesis and the orderly expression of cell cycle events.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are shown in Table 1. *C. crescentus* AE5000 (wild-type CB15) and derivative mutant strains were grown at 30°C in peptone-yeast extract broth (11) or in minimal glucose (0.2%) medium (BMG) (5) supplemented, when appropriate, with 1% Tergitol Nonidet P-40 (NP-40) plus 1 mM oleic acid or with 1 mM glycerol and 1 mM glycerol phosphate. The Tergitol NP-40 was used to solubilize the fatty acids. Cells were shifted from one medium to another by collecting them on sterile 0.45-μm membrane filters (Millipore Corp.) as described previously (Hodgson et al., submitted for publication). The double mutants and control strains were constructed as detailed in Table 1. Transductions and conjugations were performed as described by Hodgson et al. (submitted for publication).

Materials. ^{14}C-reconstituted protein hydrolysate (RPH) and ^{3}H-RPH were obtained from Schwarz/Mann. $^{32}P_i$ was from New England Nuclear Corp. Antibody to flagellin A was prepared as described previously (13). Tergitol NP-40 was from Sigma Chemical Co., and fatty acids were obtained from Supelco, except for oleate which was from Fisher Scientific Co.

Measurement of net synthesis of DNA, RNA, phospholipid, and protein. Cultures of AE6001 were grown in BMG containing Tergitol NP-40, 1 mM oleic acid, and $^{32}P_i$ (10 μCi)

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TABLE 1. *C. crescentus* CB15-derived strains used in this study

Strain	Genotype	Derivation (donor × recipient) or reference[a]
AE5000	Wild type	1
AE5168	*gpsA505* [RP4 *tet*(Am)]	1
AE5485	*flaE178*::Tn*5 fatB503 rif-501*	AE5479 × AE6006 C[b]
AE5512	*gpsA505*	AE5168 spontaneous
AE5529	*flaE178*::Tn*5 fatB503 gpsA505*	AE5485 × AE5512 T[c]
AE5571	*flaE178*::Tn*5 fatB503 fatA501*	AE5485 × AE6000 T
AE5572	*flaE178*::Tn*5 fatA501*	AE5485 × AE6000 T
AE5580	*flaE178*::Tn*5 gpsA505*	AE5485 × AE5512 T
AE5585	*flaE178*::Tn*5*	AE5485 × AE5000 T
AE5562	*flaE178*::Tn*5 fatB503*	AE5485 × AE5000 T
AE6000	*fatA501*	13
AE6001	*fatB503*	NTG[d] mutagenesis of AE5000
AE6006	*fatB503 rif-501*	AE6001 spontaneous

[a] Derivation is given in the specified reference.
[b] C, Recombination via conjugation.
[c] T, Recombination via φCr30-mediated transduction.
[d] NTG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.

for five generations to reach steady-state labeling, as described previously (2). Cells were collected on sterile filters, washed with BMG–Tergitol NP-40, and then suspended in BMG–Tergitol NP-40 plus $^{32}P_i$ (10 μCi) in the presence or absence of oleic acid (1 mM). The incorporation of label into DNA, RNA, and phospholipid was measured as previously described (Hodgson et al., submitted for publication). Total protein synthesis was measured in cultures grown in BMG–Tergitol NP-40 plus oleic acid and then labeled for 1 h with ^{3}H-RPH (10 μCi), as described previously (Hodgson et al., submitted for publication). Labeled cultures were collected on filters, washed with BMG–Tergitol NP-40, and then suspended in BMG–Tergitol NP-40 plus ^{3}H-RPH (10 μCi) with or without oleic acid (1 mM).

Immunoprecipitation of *C. crescentus* flagellins. AE6001 was grown to the mid-log phase in BMG–Tergitol NP-40 plus 1 mM oleic acid, washed, and inoculated into BMG–Tergitol NP-40 in the absence and presence of oleic acid (1 mM). At 120, 240, 360, and 480 min after the shift to BMG–Tergitol NP-40 or BMG–Tergitol NP-40 plus oleic acid, samples were pulse-labeled for 15 min with ^{14}C-RPH (3 μCi/ml). At the end of the labeling period, NaN_3 was added to 0.04 M, and the cells were collected by centrifugation and frozen. Cell extracts were prepared as described by Lagenaur and Agabian (4). The flagellins were immunoprecipitated with antiserum prepared against flagellin, using the *Staphylococcus aureus* protein A method previously described (13).

RESULTS

Growth and viability of AE6001. Mutant AE6001 (*fatB503*) was unable to grow in BMG unless unsaturated fatty acids were supplied. Since another carbon source was always present, the fatty acid supplement was not required for that purpose. The addition of Tergitol NP-40 did not support growth. The mutant remained viable in the absence of supplement (5; Fig. 1). AE6001 was adequately supplemented by all of the unsaturated fatty acids tested, including oleate, vaccenate, linolenate, elaidate, and palmitoleate. However, the saturated fatty acids tested (stearate, myristate, and palmitate) not only did not support growth but appeared to be toxic to various degrees (Fig. 1). In myristic acid-supplemented cultures the cell number increased for 4 h, and then the culture lost viability. In the presence of stearic acid, the optical density and cell number increased for 4 h before the culture began to die. Palmitic acid had a marked toxic effect in that both optical density and cell number fell sharply after 4 h. Although these cultures could not be rescued by the subsequent addition of oleic acid, the addition of oleic acid at the same time as palmitic or stearic acid greatly decreased the toxic effects of the two latter acids.

Cultures of AE6001 were examined with an electron microscope to determine whether the cell cycle was blocked upon starvation for unsaturated fatty acids. We found that by 240 min after the removal of oleic acid supplement from

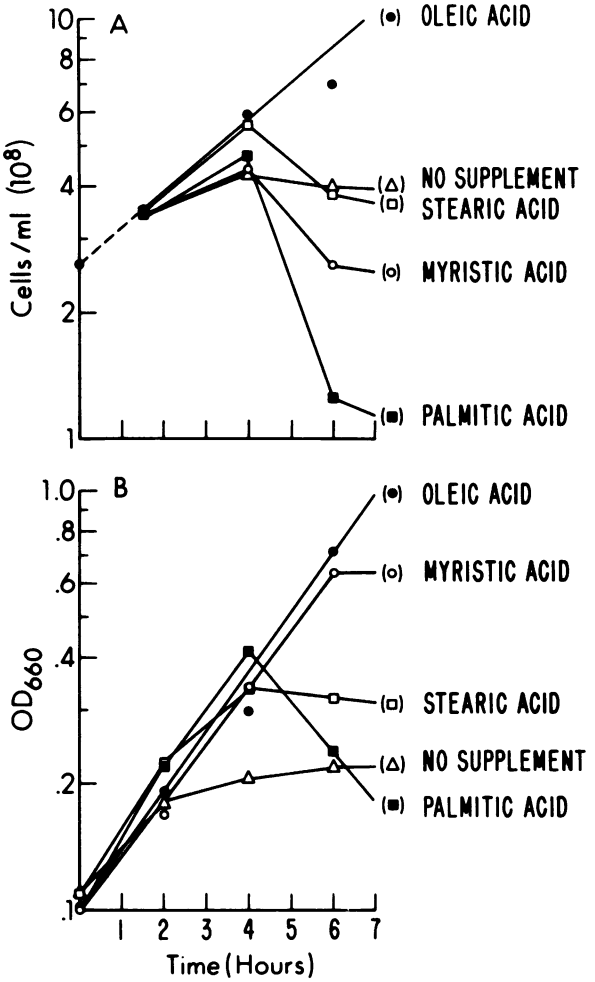

FIG. 1. Growth of AE6001 in the presence of various fatty acids. Cells were grown in peptone-yeast extract medium containing Tergitol NP-40 and 1 mM oleic acid, washed, and inoculated at time zero into peptone-yeast extract–Tergitol NP-40 without a supplement or with 1 mM oleic, myristic, stearic, or palmitic acid. (A) Viable cells per milliliter. (B) Optical density at 660 nm (OD_{660}).

log-phase cultures (the equivalent of one generation time in BMG) a large portion of cells were blocked at the predivisional stage. Predivisional cells contain a flagellum at one end and a stalk at the other end and have begun the division process. In log-phase cultures of wild-type AE5000, ca. 16% of the cells have been shown to be at the predivisional cell stage (3). The relative proportion of the cell types in cultures of AE6001 grown in the presence or absence of oleic acid was determined by analyzing electron micrographs of several hundred cells. Mid-log-phase cultures of AE6001 grown in the presence of oleic acid were found to be composed of 15% predivisional cells. At 240 min after the removal of oleic acid from AE6001, between 44 and 50% of the cells were clearly at the predivisional stage. It therefore appears that the division process is inhibited in starved cultures. The number of swarmer cells was also increased, but single-stalked cells were barely detectable, suggesting a block in the swarmer-to-stalked cell transition. The addition of oleic acid allowed the cell division cycle to resume.

Net phospholipid, DNA, and RNA synthesis in mutant AE6001 grown in the presence or absence of unsaturated fatty acids. The net synthesis of phospholipid was measured by using cells uniformly labeled with $^{32}P_i$ as described above. The cells were shifted to medium, with or without fatty acid, that contained $^{32}P_i$ at the same specific activity so that the number of counts obtained from phospholipid extracted at various times reflects both synthesis and degradation. The net phospholipid synthesis in cultures of AE6001 ceased after 2 h of oleate deprivation (Fig. 2). We have previously reported that upon starvation of AE6001 for unsaturated fatty acids there is an immediate cessation of the endogenous synthesis of both saturated and unsaturated fatty acids (5).

The net synthesis of DNA was also inhibited in starved AE6001 cultures, although it continued at a slightly slower rate than the net synthesis of DNA in supplemented cultures for an additional 2 h after the inhibition of phospholipid synthesis before the shutoff (Fig. 2). This result reflects the amount of time required for the cells in an initially mixed population to complete a round of DNA replication. In the absence of fatty acid supplement, the initiation of a new round of replication did not occur. The accumulation of predivisional cells (which represent the stage in the cell cycle when DNA replication is completed) coincident with the cessation of DNA synthesis in starved cultures provides additional evidence that a round of replication was completed and that the cell cycle was blocked. The net synthesis of RNA continued at a slightly reduced rate (Fig. 2) for several hours in the absence of unsaturated fatty acids.

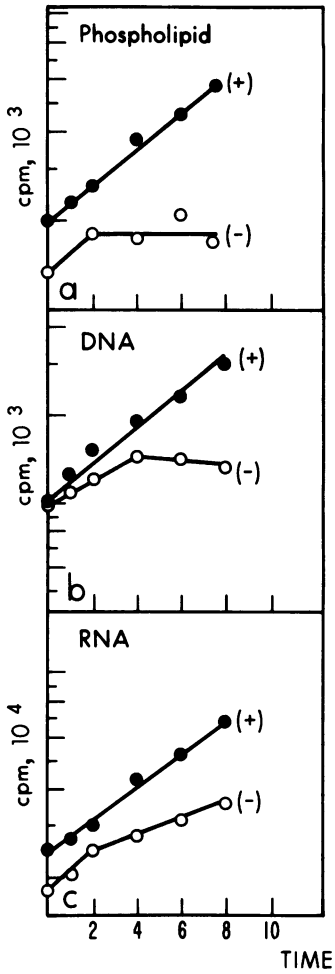

FIG. 2. Effect of fatty acid deprivation on net synthesis of phospholipid, DNA, and RNA in AE6001. Cultures were grown in oleic acid-supplemented BMG in the presence of $^{32}P_i$ for five generations. The cells were washed and suspended in medium containing $^{32}P_i$ in the presence (●) or absence (○) of oleic acid. The net synthesis of phospholipid, DNA, and RNA was determined at the times indicated as described in the text.

Effect of fatty acid starvation on the synthesis of bulk and specific proteins. Whereas net bulk protein synthesis in cultures of AE6001 was relatively insensitive to fatty acid deprivation and the consequent inhibition of phospholipid synthesis (Fig. 3), the synthesis of new flagellar proteins was inhibited after the amount of time for a round of DNA replication had elapsed. Immunoprecipitation of flagellins from cultures of AE6001 pulse-labeled at various times after the removal of fatty acid showed continued synthesis of these proteins, although at a somewhat lower level, for 4 h. The inhibition of flagellin synthesis began 2 h after phospholipid synthesis was inhibited, at approximately the same time as the arrest of DNA synthesis. Similar results were obtained when pulse-labeled cultures were immunoprecipitated with anti-hook antibody (data not shown). These results confirm previous observations that, in *C. crescentus*, DNA replication is required for flagellin and hook protein synthesis (10). Both predivisional and swarmer cells, which accumulate in unsupplemented cultures, retained their flagella after 26 h of starvation.

Epistatic effects of *fatB503* on *fatA501* and *gpsA505*. To test the prediction that a mutation in the *fatB503* gene results in a homeostatic cell cycle block upon the completion of a round of DNA replication, we constructed double mutants with other membrane mutants. We chose to determine the effect that *fatB503* would have on strains carrying either the *fatA501* allele (5; Hodgson et al., submitted for publication) or the *gpsA505* allele (1, 3). Each of these mutants has been moved into a clean genetic background, their mutations have been mapped (Hodgson et al., submitted for publication; D. Hodgson, unpublished data), and their phenotypes have been extensively characterized.

The *fatA501* allele renders the cell auxotrophic for fatty acids or biotin, blocks membrane protein synthesis, and causes cell death (apparently due to unbalanced growth) if growth is attempted in the absence of supplement (5; Hodgson et al., submitted for publication). In contrast to *fatB503*,

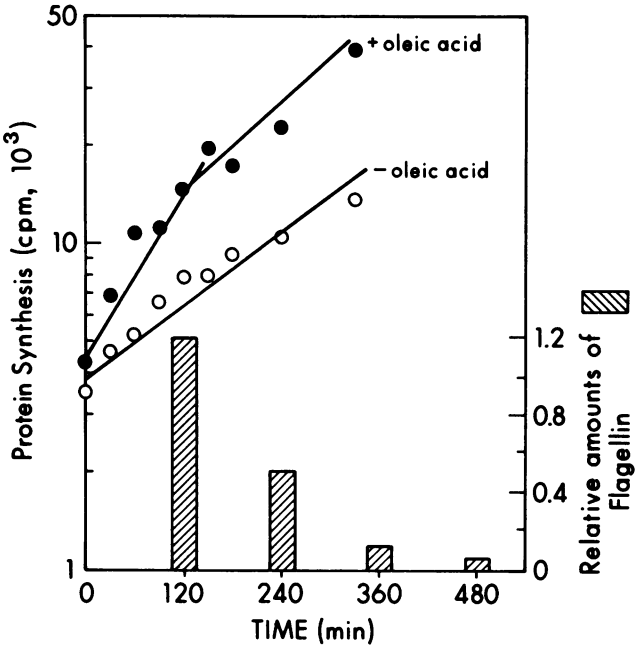

FIG. 3. Effect of fatty acid deprivation on net protein synthesis and flagellin synthesis in AE6001. Cultures were grown in BMG–Tergitol NP-40 plus 1 mM oleic acid, labeled for 1 h with ^{3}H-RPH, washed, and then placed in fresh medium with (●) or without (○) oleic acid (1 mM) in the presence of ^{3}H-RPH as described in the text. The relative amounts of flagellin A and B synthesis were determined from densitometric tracings of an autoradiogram of sodium dodecyl sulfate-polyacrylamide gel of immunoprecipitates prepared with antiflagellin antibody as described in the text. The ratio of immunoprecipitable material comigrating with the flagellins was calculated at each time point for cultures incubated in the absence and presence of oleic acid. A relative value of 1.0 represents equivalent amounts of flagellin detected in both samples.

the *fatA501* mutation results in no clear cell cycle block, and the *fatA501* cells continue DNA replication after starvation for supplement until cell death. Since *fatB503* cells are viable under starvation conditions but appear to stop the initiation of DNA replication, it is possible to examine the epistatic interactions among these phenotypes in double mutant strains. Previous experiments (Hodgson et al., submitted for publication) have demonstrated that the pleiotropic phenotype of *fatA501* (cell death, fatty acid or biotin auxotrophy, and loss of outer membrane proteins) cosegregated. Experiments with AE5662, a strain constructed by moving *fatB503* into a wild-type background, have indicated that the pleiotropic phenotype of AE6001 is due to a single lesion. Therefore it proved possible to construct a *fatA501 fatB503* double mutant (AE5571) to investigate the epistatic interaction of the *fatB503* and *fatA501* mutations. Depriving the parent strain used in these constructions [AE5585 (*fatA*$^+$ *fatB*$^+$ *gpsA*$^+$)] of oleate or biotin had no effect on cell viability (Fig. 4A). Similarly, the addition of glycerol had no effect on the growth of this strain (data not shown). These results indicate that *flaE178*::Tn*5*, which is part of the genetic background of this parent strain, had no obvious detrimental effect on cell growth. Figure 4B confirms that *fatB503* in this background requires oleate for supplementation, but cell death does not occur in its absence. Oleate or biotin had the ability to supplement the *fatA* cell in the same genetic background (Fig. 4C), whereas their absence led to cell death. The *fatA fatB* double mutant (Fig. 4E) had the same growth characteristics as the *fatB* single mutant (Figure 4B); namely, only the presence of oleate supported growth, and its absence did not lead to death. Therefore, the presence of the *fatB503* allele rescued the *fatA501* mutation from cell death upon starvation for supplement. These results indicate that *fatB* is completely epistatic to *fatA*.

The *gpsA505* mutation causes glycerol-3-phosphate auxotrophy and loss of *sn*-glycerol-3-phosphate dehydrogenase activity and, in the absence of supplement, results in a block in phospholipid synthesis (1). The effects of a block in phospholipid synthesis are the immediate cessation of DNA replication and cell death (3). The specific question we have addressed here is whether allowing cells to finish a round of DNA replication and come to rest in a homeostatic state would rescue strains carrying the *gpsA505* allele from cell death upon starvation for its auxotrophic requirement. To study the epistatic interactions between *fatB* and *gpsA*, strain AE5529 (*fatB503 gpsA505*) was constructed using the AE5585 genetic background as described in the construction of the *fatB fatA* double mutant. The mutation in *fatB* had the ability to ameliorate the glycerol-less death phenotype of the *gpsA* strain (Fig. 4D and F). However, in this case the number of survivors of glycerol starvation was increased with the length of time the cells were starved for oleate. Therefore, the epistatic effect of *fatB* was not immediate but appears to have been a reflection of the period of time required for a round of DNA synthesis to be completed.

DISCUSSION

A cell cycle mutant, AE6001, which appears to block both cell division and the initiation of DNA replication upon deprivation of exogenous unsaturated fatty acids has been analyzed. The presence of the *fatB503* allele in strain AE5001 was previously shown to cause an immediate cessation of [^{14}C]acetate incorporation into both saturated and unsaturated fatty acids upon starvation of AE6001 for exogenous unsaturated fatty acids (5). This block in total fatty acid synthesis was followed by a cessation of phospholipid synthesis. Supplementation of AE6001 with unsaturated fatty acids allowed their incorporation, unaltered, into phospholipid (5). The fatty acid palmitate was also incorporated into phospholipid (5), although attempted growth on this or other saturated fatty acids was lethal. The addition of exogenous unsaturated fatty acids, but not saturated fatty acids, allowed endogenous fatty acid synthesis to occur. We have previously demonstrated that there is no change in the fatty acid and phospholipid composition of this mutant under conditions of fatty acid starvation and that this composition is the same as that found in wild-type cells (5). The phenotype of this fatty acid auxotroph is not consistent with a simple defect in fatty acid synthesis but appears to reside in the coupling of lipid metabolism to cell cycle events in *C. crescentus*.

The sequence of cell cycle events which are altered upon fatty acid starvation of strains carrying the *fatB503* allele is shown schematically in Fig. 5. After the cessation of fatty acid and phospholipid synthesis, a block in net DNA synthesis occurred after an amount of time had elapsed which is equivalent to that required to complete a round of DNA replication. This suggests that a block in phospholipid synthesis in this mutant results in the inhibition of the initiation of DNA replication. This conclusion is supported by the observation that cultures of AE6001 accumulated predivisional cells which carry a newly formed flagellum, suggest-

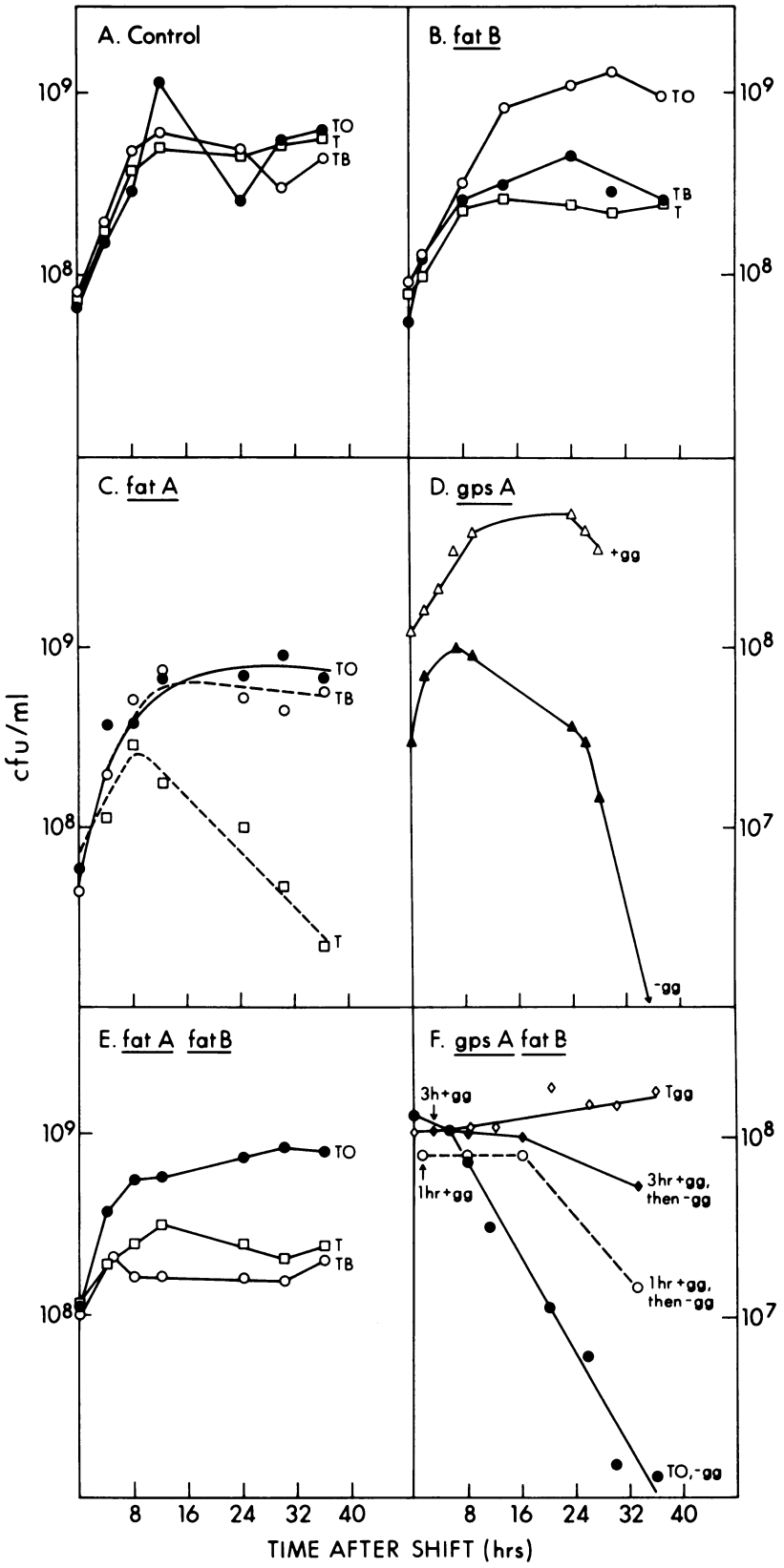

FIG. 4. Growth curves of a glycerol auxotroph, AE5580 (*gpsA505*), and two fatty acid auxotrophs, AE5572 (*fatA501*) and AE5485 (*fatB503*), showing the epistatic effect of double mutants carrying the *fatA501 fatB503* alleles and the *gpsA505 fatB503* alleles AE5571 and AE5529, respectively. (A, B, C, and E) Viable cells per milliliter (CFU per milliliter) as a function of time for cultures grown in BMG–Tergitol NP-40 plus 1 mM oleic acid, washed on filters, and then shifted to BMG containing Tergitol NP-40 (T), oleic acid (1 mM) plus Tergitol NP-40 (TO), or biotin (2 mM) plus Tergitol NP-40 (TB). (A) Viable counts obtained for the parent strain AE5585 which carries *flaE*::Tn*5*. (D and F) Viable cells as a function of time for cultures grown in BMG–Tergitol NP-40 plus 1 mM oleic acid containing glycerol and glycerol phosphate PO_4 (gg), washed on filters, and then shifted to BMG–Tergitol NP-40 containing either glycerol (1 mM) and glycerol phosphate (1 mM) or oleic acid (1 mM). As indicated in (F), two cultures were first shifted to BMG–Tergitol NP-40 plus glycerol and glycerol phosphate for 1 and 3 h, respectively, before being washed on filters and then shifted to BMG–Tergitol NP-40. AE5529 suspended in the presence of BMG–Tergitol NP-40 plus 1 mM oleic acid and glycerol and glycerol phosphate gave the same increase in CFU per milliliter as AE5580 (D) in the presence of glycerol and glycerol phosphate.

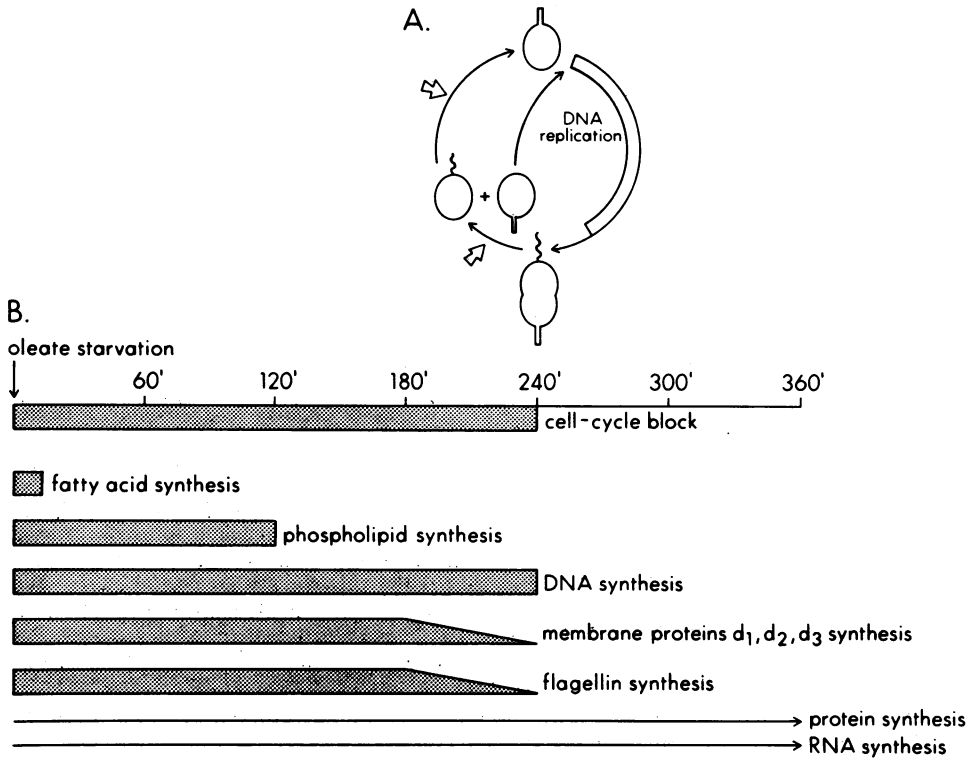

FIG. 5. Schematic of the *C. crescentus* cell cycle (A) and a diagram (B) of a mid-log-phase culture of AE6001 showing the time after the shift to oleate-starved medium in which fatty acid synthesis (5), phospholipid synthesis, DNA synthesis, and outer membrane protein d1, d2, and d3 synthesis (13) were blocked. The generation time of this culture in BMG–Tergitol NP-40 plus 1 nM oleic acid was 240 min. The open arrows (A) indicate the stages in the cell cycle which were blocked.

ing that DNA synthesis had occurred and allowed synthesis of the flagellar components. It has been demonstrated that flagellin and hook proteins require the replication of the chromosome for their synthesis (10, 14). We show here that once cultures of AE6001 were blocked at the predivisional stage they no longer synthesized flagellins, providing confirmation that these cultures are unable to resume the cell cycle and to initiate DNA replication. Similarly, the three outer membrane proteins which are dependent on DNA replication for their differential synthesis were also inhibited after the block in the cell cycle (13). Electron micrographs of blocked cultures also showed, in addition to predivisional cells, the presence of a small proportion of swarmer cells. These cells were probably present because they were unable to initiate DNA replication and differentiate into stalked cells.

Direct evidence that the mutation in AE6001 lies in a link between lipid metabolism and the normal progression of the cell cycle comes from the observation that the predivisional cell, which retains its flagellum and is motile, is unable to divide unless unsaturated fatty acids are added to the culture. An analysis of double mutants carrying the *fatB503* allele supports the conclusion that unsupplemented cultures come to rest at a homeostatic state upon completion of chromosome replication but before cell division. We have shown previously that the *fatA* gene is required for the regulation of fatty acid metabolism and that the attempted growth of *fatA* mutants in the absence of unsaturated fatty acid supplement leads to cell death. In starved *fatB503* cultures we have shown that fatty acid synthesis is blocked and that this block occurs immediately (Fig. 5; 5, 13). The presence of the *fatB503* allele in mutants carrying *fatA501* rescues cultures from cell death in the absence of supplement, without any prior activation of the *fatB503* phenotype. Fatty acid synthesis continues in *fatA501* cultures starved for supplement, but membrane protein synthesis is blocked (Hodgson et al., submitted for publication). It is possibly this unbalanced growth that leads to cell death. One explanation for the rescue of *fatA* from cell death could be that *fatB* is preventing the cultures from going into unbalanced growth. Alternatively, it could be that the death of *fatA* cultures is due to the accumulation of a toxic product from deregulated fatty acid synthesis and that rescue by the presence of *fatB* is due to inactivation of the pathway that leads to the accumulation of this putative toxic product.

In addition, the conclusion that AE6001 (*fatB503*) is able to complete a round of DNA replication and thus remain viable upon fatty acid starvation is supported by the observation that the presence of the *fatB503* allele can rescue a *gpsA* mutant from cell death. Strains carrying the *gpsA505* allele lack glycerol-3-phosphate dehydrogenase activity and are dependent on exogenous glycerol phosphate for growth (1). Starvation for glycerol phosphate was shown to cause both the immediate cessation of DNA synthesis and cell death (3). An analysis of the *gpsA505 fatB503* double mutant has shown that, if the cultures were allowed to grow for increasing amounts of time in the presence of glycerol phosphate but in the absence of oleate before the glycerol phosphate was removed, then the number of viable cells increased proportionally to the amount of time the culture was incubated in the presence of glycerol phosphate and in the absence of oleate. The simplest conclusion from this

experiment is that in the presence of glycerol phosphate, but in the absence of oleate, increasing numbers of cells in the cultures were allowed to complete a round of DNA replication and come to rest in a homeostatic state, thus rescuing the cultures from glycerolless death. This result further suggests that the loss of viability in the glycerol auxotroph is due to unbalanced growth resulting from the immediate cessation of DNA synthesis upon glycerol phosphate starvation.

We have shown previously that, in *C. crescentus*, membrane lipid biosynthesis is tightly coupled to the expression of cell cycle events (1, 3, 7, 13; Hodgson et al., submitted for publication) and that phospholipid biosynthesis varies as a function of the cell cycle (6). The identification of a fatty acid-dependent cell cycle mutant provides additional evidence for a regulatory role for membrane lipid metabolism in the complex events which control cell division and differentiation in *C. crescentus*. This mutant also identifies a point in the cell cycle not previously represented by existing *Caulobacter* cell cycle mutants. Other mutants which block the initiation of DNA synthesis go on to form filaments (8), presumably because they are able to continue membrane assembly. It has been demonstrated in *C. crescentus* that DNA chain elongation and completion are required for cell division (9). A similar phenotype is seen in yeast cell cycle mutants blocked in DNA replication, again because the replication of DNA and the expansion of budding yeast cell surface are on independent pathways in the cell cycle (12). The yeast cell cycle and the *Caulobacter* cell cycle are similar in that they both contain several independent pathways of events. The ability of the cell to stop growing in a balanced state requires coordination of these independent pathways. The *fatB503* mutant blocks both the initiation of DNA synthesis and membrane assembly in a balanced fashion and thus may represent a lesion in a junction involved in coordination of DNA replication and membrane biogenesis.

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