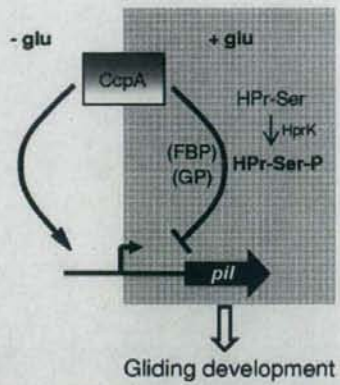

FIG. 8. A dual (positive and negative) role for CcpA in *C. perfringens* gliding development. The present cartoon depicts one hypothetical model that might explain our actual knowledge of the gliding phenotypes of CcpA-proficient and CcpA-deficient *C. perfringens* strains grown in the presence and absence of sugar supplementation. In the absence of glucose (−glu; left) or other catabolite-repressing sugars, CcpA by itself might be able to bind to the positive regulatory regions of genes (*pil*) involved in TFP expression (i.e., *pilT* and *pilD*), producing a positive effect on transcription and hence stimulating gliding proficiency. In support of this view, it has also been reported that in vitro and in vivo, CcpA-DNA mediated interactions do occur in the absence of added sugars (20, 29, 35). In the presence of catabolite-repressing amounts of glucose (right), the phosphotransferase enzyme of the sugar-specific phosphotransferase system Hpr-Ser would be phosphorylated by HprK (35). Hpr-Ser-P_i would bind to CcpA, and the newly formed Hpr-Ser-P_i::CcpA complex would interact with repressor sites located on the regulatory regions of *pil* (*pilT* and *pilD*) and therefore interfere with gliding proficiency. Also shown in the picture is the possibility that the coeffectors fructose 1,6-bisphosphate (FBP) and glucose 6-phosphate (GP) would function as adjunct corepressors to enhance and to fine-tune the response of CcpA to the metabolic needs of the cell (35, 43). Another possibility (an indirect effect of CcpA) that is not illustrated in this model is that CcpA would dually regulate an unidentified factor responsible for switching on and off the expressions of *pil* genes.

grown in TY broth without sugar supplementation. As observed in Fig. 7C, there are unambiguous down-regulations of *pilT* and *pilD* expression in the cultures deficient in CcpA production. This positive role of CcpA in TFP expression (Fig. 7C) and social gliding proficiency (Fig. 6A and 7B) in the absence of sugar supplementation and its opposite (negative) effect on the same social behavior (gliding motility) under conditions of carbon catabolite regulation (Fig. 6, presence of sugar) suggest a novel, dual (activating and repressing) role for CcpA in regulating *C. perfringens* gliding motility (Fig. 8).

DISCUSSION

Our current study shows several significant contributions toward the understanding of the physiology and regulation of TFP-dependent gliding motility in *C. perfringens*. First, we extended the analysis to a total of 17 different *C. perfringens* strains isolated from diverse infections (diarrhea, food poisoning, myonecrosis) produced not only in human beings but also from animal origins (Table 1). Interestingly, all the analyzed strains exhibited active proficiencies in social gliding motility on agar surfaces (Fig. 1 and data not shown). These results significantly consolidate and strengthen the idea that gliding motility is an intrinsic property of pathogenic *C. perfringens*, regardless of its origin of isolation (41).

Our understanding of the environmental and metabolic factors that control surface-associated translocation in pathogenic bacteria is very limited. Precisely, the main contribution of our work is the demonstration that carbon catabolite repression (20, 39, 43, 50) regulates social gliding motility in *C. perfringens*. In fact, all the surveyed isolates exhibited social gliding motility on BHIA plates (with no glucose supplementation) but not on TGYA medium which contained 2% glucose, suggesting that glucose is capable of inhibiting social gliding motility. The removal of glucose from TGYA allowed the cells to exhibit social motility, while the addition of glucose in BHIA resulted in the inhibition of gliding motility, confirming that glucose plays a crucial role in inhibiting gliding motility (Fig. 1 and data not shown).

In addition to glucose, gliding motility was also inhibited by other rapidly metabolized sugars, such as fructose, lactose, sucrose, and galactose (Fig. 3). This finding confirmed that the repression of gliding in *C. perfringens* was due to a general process of carbon catabolite repression (43). Interestingly, two complex carbohydrates, raffinose and starch, behaved differently from the single sugars: raffinose did not inhibit motility at any of the assayed concentrations, and starch inhibited gliding only at concentrations higher than 2% (Fig. 3 and data not shown). These results are consistent with previously reported findings that other social behaviors present in *C. perfringens*, such as sporulation and enterotoxin (CPE) production, were also repressed by rapidly metabolized single sugars, such as glucose and lactose (28, 42), while the complex carbohydrates raffinose and starch were found to induce both events (17, 18). The correlation between carbon catabolite repression of sporulation and surface-associated motility suggests that the two social processes might share, at least in part, a common regulatory network (Fig. 9).

We demonstrate that carbon catabolite repression of gliding motility in *C. perfringens* occurs through the repression of at least two genes involved in TFP production and functionality, namely, *pilD* and *pilT*. As observed in Fig. 5, the addition of 1% glucose to growing cultures of the gliding-proficient reference strains 13 and SM101 resulted in dramatic decreases in *pilD-gusA* and *pilT-gusA* expression. The maximum reduction in transcription due to the added glucose occurred approximately after 24 h of growth, which is consistent with the observation that gliding motility on agar plates begins only after 18 to 20 h of growth (Fig. 4 and data not shown).

In low-G+C-content, gram-positive bacteria, carbon catabolite regulation is under the control of the key transcription factor CcpA (carbon catabolite protein A), a member of the LacI-GalR family of transcriptional regulators (43). In the better-known cases of CcpA-mediated carbon catabolite regulation (i.e., in *Bacillus subtilis* and *Lactococcus lactis*), a complex and sophisticated signaling network is present (20, 29, 40, 50). Basically, the CcpA-dependent regulatory network utilizes sugar transporters, glycolytic enzymes, and an ATP-dependent, metabolite-activated protein kinase (HprK) and two small HprK target proteins: the phosphotransfer protein Hpr of the phosphotransferase system and the Hpr homologue Crh (35, 43). Moreover, a central role has been reserved for CcpA,

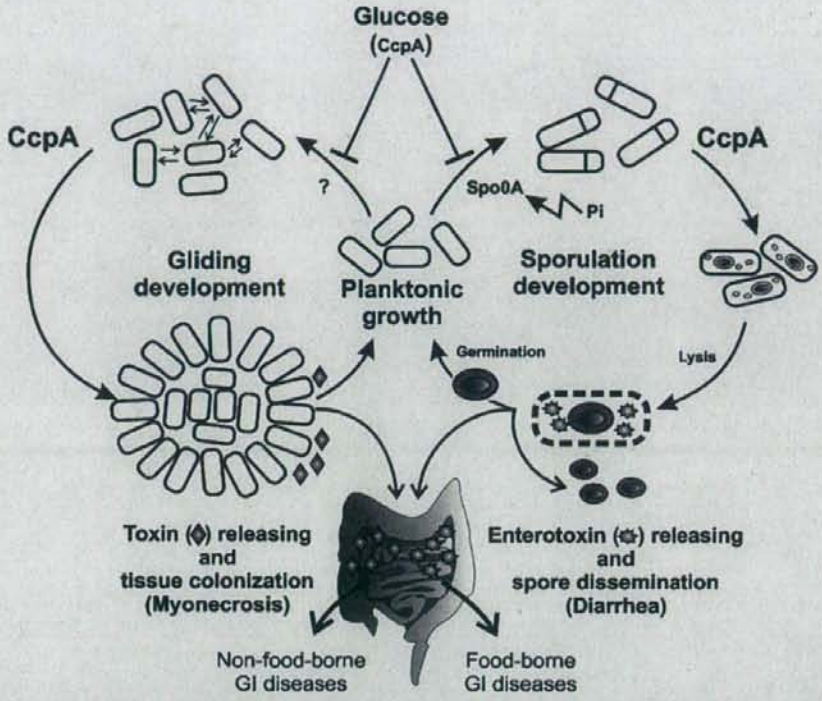

FIG. 9. A workable model linking carbon catabolite regulation of social behaviors (gliding, sporulation, and toxin production) with disease progression in *C. perfringens*. In this hypothetical but realistic scenario, toxigenic, vegetative *C. perfringens* cells that reach the lumen of a human or animal gastrointestinal (GI) tract, where the basal luminal concentrations of glucose are normally lower than 0.5% (12), have the possibility of undergoing at least two different differentiation pathways: sporulation and/or gliding development. In the first case, the activation of the key transcription factor Spo0A by inorganic phosphate (P_i) present in the intestinal lumen triggers spore morphogenesis and enterotoxin (CPE) production (28). In the case of gliding development (left), unknown signals that might be linked to cell-cell and cell-surface interactions (double arrows) orchestrate the spatial and temporal organization of the cells to the onset of gliding. The progression of either developmental program (sporulation or gliding) would not exclude the occurrence of the other alternative pathway: sporulation and CPE production would take place in the lumen of the GI tract while gliding motility and vegetative toxin synthesis (i.e., collagenase production) would take place in association with the intestinal mucosa. The key role of glucose (representing the occurrence of CcpA-mediated carbon catabolite regulation when the level of the sugar is at least 1%) as a repressor of sporulation (25, 28) and gliding (this study) development is indicated. This regulatory blockage derives from the inhibition of enterotoxin (25, 28) and vegetative-linked toxin (11, 42) production in *Clostridium* spp. The novel role of CcpA as an activator of sporulation (42) and gliding proficiency (this study) is also shown. The development of inhibitors (e.g., monosaccharide analogs) that block the onsets of gliding and/or sporulation or antagonists that interfere with the positive role of CcpA on toxin production would contribute to combating the outbreak and dissemination of clostridial diseases.

which binds to DNA sequences (*cis*-acting replication element sites) present on the regulatory regions of its target genes (20, 29, 35, 43). For the activation of CcpA binding to the *cis*-acting replication elements, it is necessary, although not essential (20, 29, 35), for CcpA to bind to the phosphorylated forms of Hpr and/or Crh produced by HprK (35, 43). In *C. perfringens*, orthologs of *ccpA*, *hpr*, and *hprK* (but not *crh*) are present on the chromosomes of all the sequenced strains, suggesting that the basic elements for CcpA-mediated carbon catabolite regulation are present in this pathogen (reference 43 and data not shown). In fact, we demonstrated that the repression of *C. perfringens* gliding motility by glucose was mediated, in large part, by the action of CcpA. As observed in Fig. 6, the inactivation of *ccpA* significantly restored gliding proficiency (Fig. 6A) and *pil* expression (Fig. 6B) in the presence of glucose. The reversion to the gliding-deficient phenotype of the *ccpA* mutant strain in the presence of glucose was obtained after the introduction (by DNA electroporation) of the plasmid pIH100, harboring a wild-type copy of *ccpA*, which provided direct genetic evidence supporting the strong linkage between CcpA expression and carbon catabolite repression of gliding motility in *C. perfringens*. These results suggest that CcpA could act as a transcriptional regulator of TFP biosynthesis genes. However, this effect might be indirect since no putative *cre* sites have been identified in any of the TFP biosynthesis genes analyzed so far (data not shown). It might be possible that other *cre*-like consensus sequences, different from the ones reported for *Bacillus* and other low-G+C-content, gram-positive bacteria, exist in clostridia (20, 42, 43). Another possibility is that, apart from CcpA, an unidentified intermediate factor might be involved in regulating TFP gene expression. This suggestion received support based on the observation that the *ccpA* mutant strain was not able to restore, in the presence of sugar supplementation, full gliding proficiency and *pil* expres-

sion as the levels reached that of the wild-type strain in the absence of added sugars (Fig. 6 and data not shown).

A final and unexpected finding of our study was the observation that, in the absence of added sugar, CcpA has a positive role in gliding motility. As observed in Fig. 6, in the absence of added glucose, the *ccpA* mutant strain glided on the agar plate to a lesser extent than the isogenic wild-type strain. As observed in Fig. 7B, the wild-type strain (CcpA proficient) reached a maximum speed of gliding of 630 to 670 $\mu m\ h^{-1}$, while its isogenic *ccpA* derivate (CcpA deficient) reached a maximal speed of gliding of 220 to 250 $\mu m\ h^{-1}$ only. Two observations argue strongly for a positive role for CcpA in gliding development: first, the *ccpA* mutant strain did not show any growth defect on liquid medium, reaching essentially the same final OD and viable-cell number as the wild-type strain (Fig. 7A); furthermore, the results for the initial phase of colony growth (before the onset of gliding) were very similar for both the $ccpA^+$ and *ccpA* strains (Fig. 7B and data not shown). This hypothesis was reinforced by the demonstration that CcpA production was required for efficient expression of *pilT* and *pilD* in growth media without sugar supplementation (Fig. 7C). These findings indicate that CcpA has a dual role in controlling gliding motility in *C. perfringens* (Fig. 8). In the presence of rapidly metabolized sugars (e.g., glucose), CcpA has a negative role on the onset of gliding, an effect that is partly mediated through repression of *pilT* and *pilD* expression (Fig. 6). In the absence of added sugars, CcpA switches to a positive role on gliding, a novel property that is uncovered by the deficient gliding phenotypes and poor *pilT* and *pilD* expression levels of CcpA-deficient cells cultured in the absence of added glucose (Fig. 7). In agreement with our finding, a similar positive role for CcpA in spore formation and *cpe* expression under conditions without catabolite regulation (in the absence of added sugars) has been reported for *C. perfringens* (42).

Excess glucose in the environment of *C. perfringens* not only affects stationary phase phenomena, such as sporulation-linked CPE production (25, 28, 42) and gliding motility (this study), but can also act as a catabolic repressor of collagenase production during vegetative growth (42). Moreover, in the other intestinal pathogenic *Clostridium* bacterium *C. difficile*, glucose represses toxin production (11). Importantly, within the context of the development of a clostridial infection, it is plausible to envision that proficiency in gliding associated with toxin production and tissue damage would contribute to the progression of the infectious process (Fig. 9). Luminal glucose concentrations in the small intestines of mammals are in the range of 0.006% to 0.4% (12). Interestingly, in our study the catabolite repression of gliding motility by glucose was concentration dependent; surface motility was observed only when the glucose concentration was less than 0.5% (Fig. 2). This finding opens the possibility that gliding willingly would occur during the course of a clostridial infection (Fig. 9). We are just grasping the regulatory network of surface-associated motility in pathogenic clostridia, and the understanding of how carbon catabolite repression inhibits known and potential virulence processes (sporulation, toxin production, and gliding motility) in *C. perfringens* (6–8, 21, 26, 36) will contribute to preventing and combating clostridial diseases (Fig. 9).

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