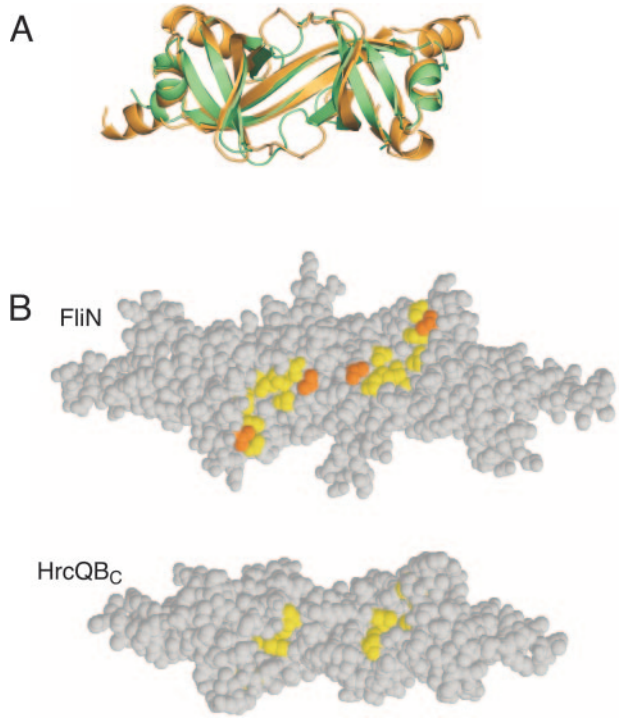

FIG. 10. Comparison of FliN and HrcQB$_C$ structures in the region of the hydrophobic patch. (A) Ribbon diagram showing the FliN dimer (gold) superimposed on half of the HrcQB$_C$ tetramer (green). The view is along the twofold dimer axis, looking onto the hydrophobic patch. The largest differences between FliN and HrcQB$_C$ occur in the loops connecting β2 and β3 (residues 107 to 114 of FliN), which in FliN frame the hydrophobic patch. (B) The hydrophobic patch is larger in FliN than in HrcQB$_C$. The modeled FliN tetramer and the crystal structure of the HrcQB$_C$ tetramer are shown, and the hydrophobic residues of the patch are yellow or orange. The view is along the twofold axis of the tetramer (as in Fig. 9A). Orange indicates a valine residue (Val130 in *T. maritima*, corresponding to Val113 in *E. coli*) that was mutated to aspartic acid to test the functional importance of the patch.

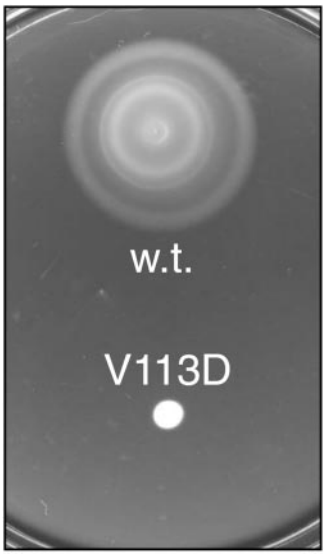

FIG. 11. Mutation of a residue in the hydrophobic patch eliminates swarming in soft-agar tryptone plates. *E. coli* strain DFB223, null for *fliN*, was transformed with plasmids that encode either wild-type *E. coli* FliN (w.t.) or FliN with the mutation V113D. The plate was inoculated with 2 μl of saturated overnight cultures and incubated at 32°C for 8 h.

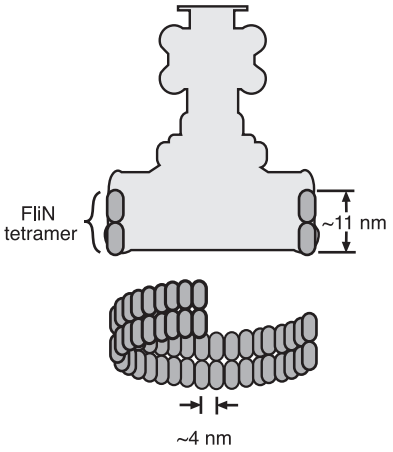

FIG. 12. Model for the arrangement of FliN tetramers in the C ring. The orientation shown for the FliN tetramers is suggested by en face electron micrographs of the C ring that showed a ~34-fold subunit structure and ~4-nm subunit spacing (69, 79).

hydrophobic patch mutants should reveal whether they are defective in flagellar export or in other steps of assembly. Hydrophobic surface features that look similar are found on some small heat shock proteins that function as chaperones (34, 74), and one possibility is that FliN functions as a cochaperone for flagellar export by providing docking sites for chaperone-cargo complexes. The large structural differences between FliN and HrcQB$_C$ in the region of the hydrophobic patch (Fig. 10) are consistent with such an export function, because the virulence factor export apparatus acts on a different set of substrates and utilizes different chaperones (15).

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REFERENCES

1. **Aizawa, S.-I.** 2001. Flagella, p. 155–175. *In* Bacterial structure. Academic Press, New York, N.Y.
2. **Aldridge, P., J. E. Karlinsey, and K. T. Hughes.** 2003. The type III secretion chaperone FlgN regulates flagellar assembly via a negative feedback loop containing its chaperone substrates FlgK and FlgL. Mol. Microbiol. **49:**1333–1345.
3. **Alfano, J. R., and H. S. Kim.** 1997. Evidence that the *Pseudomonas syringae* pv. *syringae hrp*-linked *hrmA* gene encodes an Avr-like protein that acts in an *hrp*-dependent manner within tobacco cells. Mol. Plant-Microbe Interact. **10:**580–588.
4. **Bennett, J. C. Q., and C. Hughes.** 2000. From flagellum assembly to virulence: the extended family of type III export chaperones. Trends Microbiol. **8:**202–204.
5. **Bennett, J. C. Q., and C. Hughes.** 2001. Substrate complexes and domain