## **Supporting Information**

Clarke et al. 10.1073/pnas.0806786105

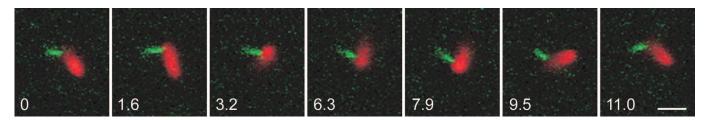
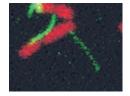


Fig. S1. Rotation of a cell tethered to the substratum by an F-pilus. The figure contains frames 7 to 14 from Movie S10, showing one complete rotation of the cell. (Frame 10 was omitted because the cell was out of focus.) Time is shown in seconds after the first frame. (Scale bar, 2  $\mu$ m.)



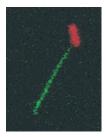
**Movie S1.** Extension of F-pili. This cell extends a pilus that reaches a length of 5  $\mu$ m before beginning to retract. During extension, the cell-proximal portion of the pilus is poorly labeled by fluorescent phage, but the labeling becomes uniform during retraction. A second F-pilus extends briefly then retracts. The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S1 (MOV)



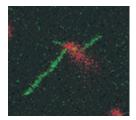
**Movie S2.** Retraction of an F-pilus. This cell has an F-pilus that is 4  $\mu$ m in length when observation begins; the pilus retracts completely. The frames are separated by 2 s; movie plays at 25× actual speed.

Movie S2 (MOV)



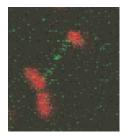
Movie S3. Stalled retraction of a long F-pilus. This F-pilus reaches a length of 9  $\mu$ m. It is extending as the time series begins, but switches to retraction within a few frames, as indicated by the increased cell-proximal labeling of the filament by R17. Retraction, although initially rapid, gradually slows and has almost stalled by the end of the time series (13 min), probably owing to the increasing accumulation of bacteriophage. The frames are separated by 1.6 s; the movie plays at 25× actual speed.

Movie S3 (MOV)



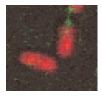
**Movie S4.** Independent regulation of F-pili on the same cell. This cell extends three pili, which grow and retract asynchronously. The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S4 (MOV)



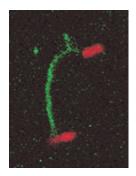
**Movie S5.** Cell-cell contact resulting from F-pilus retraction. An HfrH cell extends an F-pilus whose tip contacts another HfrH cell. Retraction of the pilus draws the cells together, rotating and flipping the dividing cell during the process. The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S5 (MOV)



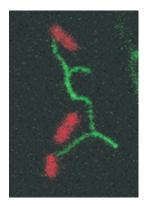
**Movie S6.** Cell-cell contact resulting from F-pilus retraction. Two HfrH cells are already linked by an F-pilus when observation begins; retraction draws the two cells together. After a brief period of contact, the two cells separate. The frames are separated by 1.6 s; the movie plays at 25× actual speed.

Movie S6 (MOV)



**Movie S7.** Supercoiling during F-pilus extension. The distal portion of a growing F-pilus contacts and binds to another F-pilus. Further extension results in supercoiling, indicating the pilus is rotating as it extends. The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S7 (MOV)



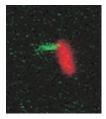
**Movie S8.** Supercoiling during F-pilus extension and retraction. A growing F-pilus is constrained by the binding of its distal portion to an F-pilus on another cell. As extension continues, a supercoil appears. The supercoiled segment elongates as the pilus grows, then shortens as it retracts. (Retraction is indicated by increased phage binding along the cell-proximal portion of the filament.) The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S8 (MOV)



**Movie S9.** Supercoiling during F-pilus extension. The tip of an extending F-pilus adheres to the substratum, and further growth leads to supercoiling. The frames are separated by 2 s; the movie plays at 25× actual speed.

Movie S9 (MOV)



Movie S10. Rotation of an HfrH cell tethered to the substratum by an F-pilus. The cell shown here and in Fig. S1 is tethered to the coverslip by a short, heavily labeled F-pilus. The cell is rotating at a slow and diminishing rate that correlates well with the retraction behavior of a heavily labeled F-pilus, illustrated in Movie S3. While these data support F-pilus rotation, accurate quantitative measurements of unimpeded rotation rates will require equipment with much higher temporal resolution than that available for the present study. The frames are separated by 1.6 s; the movie plays at 25× actual speed.

Movie S10 (MOV)