Supporting Information

Direct Observation of Type 1 Fimbrial Switching
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Strains and plasmids

The bacterial strains used in this study are derivatives of *E.coli* K-12. For experiments without SeqA foci we used strain ASC129, and for experiments with SeqA foci we used strain ASC215. Intermediate strain AAEC112 (MG1655 3′*fimE*-5′*fimA* Δ*sacB*-Kan<sup>R</sup>) and plasmid pIB315 (Cm<sup>R</sup>, temperature sensitive) containing the *fimE*-fimD genes derived from MG1655 (Blomfield et al., 1991a) were used in allelic exchange (Blomfield et al., 1991b) to construct a *fimA*-gfpmut2 fusion in the genome at *fim*. The gfpmut2 fusion (Cormack et al., 1996) in the resultant strain (ASC129) is cloned into the *HpaI* site of *fimA*.

Into vector pSAV047 (Amp<sup>R</sup>, pBR322 ori; *rop*; a generous gift from S.Alexeeva) seqA gene from pGAP40 (kindly provided by T.Brendler (Brendler et al., 2000)) is fused to the N-terminus of mCherry (Shaner et al., 2005) fluorescent protein under the control of a repressed trc down promoter resulting in plasmid pASC215. Strain ASC215 is ASC129 transformed with pASC215.

Growth conditions

The media used for cloning and allelic exchange included LB Lennox broth (Difco) and LB Lennox agar (Difco). Sucrose agar is LB agar without sodium chloride and supplemented with 6% sucrose (Sigma). Whenever necessary chloramphenicol, kanamycin, and ampicilin was supplemented to the media to a final concentration of 30, 25 and 100µg/mL respectively. Cells are grown at 28, 37 and 42ºC as required (Blomfield et al., 1991b).

For microscopy experiments, EZ Defined Rich Medium (Teknova) and MOPS medium (Teknova) supplemented with 0.4% sodium succinate (Sigma) was used. Whenever SeqA was visualized, 100µg/mL ampicilin (Sigma Aldrich) was added to the medium, no IPTG was added. For cell immobilization and microcolony growth, MOPS agar slab was made by mixing hot 2% agaroseMP (Roche) solution to concentrated medium as mentioned above. Cells were grown at 37ºC. All liquid cultures were grown aerobically. To make silanized coverslips, Repel Silane was purchased from Amersham Biosciences.

Microscopy

Imaging is performed with a Nikon Eclipse TE2000 inverted microscope equipped with a 37ºC incubation chamber, automated stage (Marszauser) and CCD camera (Coolsnap, Roper Scientific). Phase contrast and GFP fluorescence images were taken automatically by imaging software Metamorph (RoperScientific) with 100X magnification objective lens every 4 minutes. For SeqA experiments, an additional magnification of 1.5X was used. Light from a xenon lamp (Lambda LS) is filtered by GFP filters (Nikon) and HCRed filters (Chroma) for GFP and mCherry detection. mCherry fluorescence images were taken every other time step. Depending on the growth medium and the experimental purpose, typical experiments lasted from 5 to 16 hours.

Data analysis

Elementary image manipulation and viewing was done with Metamorph, ImageJ and IrfanView. Cell fluorescence quantification and lineage tree construction was performed with Schnitzcell, a program written in MATLAB, kindly provided by M. Elowitz (Caltech) (Rosenfeld et al., 2005). The mean fluorescence value is calculated for each cell and at each experimental timestep. It is calculated as the sum of all (background-corrected) fluorescence values of the pixels that correspond to the cell, divided by the total number of pixels.
**Movie S1. ON switching in a growing microcolony**
This film shows 216 minutes (54 frames of overlaid phase contrast and fluorescence images) of microcolony growth from a single OFF cell condensed to 18 seconds. Images were taken every 4 minutes. Multiple switching ON events were indicated by patches of fluorescent cells (green cells). Medium is defined rich.

**Movie S3. ON switching in a growing microcolony**
This film shows 192 minutes (48 frames of overlaid phase contrast and fluorescence images) of microcolony growth from a single OFF cell condensed to 16 seconds. Images were taken every 4 minutes. An ON switching event was indicated by patches of fluorescent cells (green cells). Medium is defined rich.

**Movie S2. OFF switching in a growing microcolony.**
This film shows 228 minutes (57 frames of fluorescence images) of microcolony growth from a single ON cell condensed to 19 seconds. Images were taken every 4 minutes. Multiple switching OFF events were indicated by gradual decrease of fluorescence in some cells. Medium is defined rich.

**Movie S4. OFF switching in a growing microcolony.**
This film shows 168 minutes (42 frames of fluorescence images) of microcolony growth from a single ON cell condensed to 14 seconds. Images were taken every 4 minutes. Multiple switching OFF events were indicated by gradual decrease of fluorescence in some cells. Medium is defined rich.

**Figure S1. Exponential growth of microcolonies**
The number of cells within a microcolony increases exponentially until the end of the experiment. The data correspond to 4 microcolonies growing on defined rich agar surface in 2 different experiments (colored lines). Black straight lines are exponential fits to the experimental data. These data produced similar growth rates as bulk liquid experiment (mean doubling time = 24.5 minutes and 24.7 minutes respectively).
Figure S2. Determination of GFPmut2 maturation time
The mean fluorescence level for 4 cells was followed in time after IPTG addition (t=0). The gray square at t=0 represents the average background fluorescence level for non-induced cells (9.1 ± 1.7 \times 10^{-3} \text{ a.u}). The straight lines are linear regression lines over experimental data (symbols). The average time after induction at which fluorescence is detected is 5.7 ± 0.7 minutes. This time includes the GFP maturation time. Strain: *E. coli* MG1655 bearing plasmid pTHV038 (a generous gift from T. den Blaauwen). Growth conditions: MOPS medium supplemented with succinate at 37°C.

Figure S3. OFF switching in a growing microcolony
The full arrow indicates the switching cell at the time close to its birth. Both daughters have lowered fluorescence (two open arrows). Subsequently, one daughter restores fluorescence (top daughter lineage- grey line), while the other monotonously decreases fluorescence by dilution (lower daughter lineage- black line). This
expression pattern with a transient decrease mirrors the transient expression peak for ON switching after \textit{fim} replication. The pattern is explained by the epigenetic inheritance of a diluted (reduced) GFP concentration after switching one of the two \textit{fim} copies, with the genetic inheritance by one daughter of one ON \textit{fimS}, and one OFF \textit{fimS} by the other daughter. Green and red lines represent the lineages that stay in the ON state. The growth medium is MOPS supplemented with succinate.

![Figure S4](image)

**Figure S4. The effect of Dam on the \(\beta\)-galactosidase produced by a FimB-LacZ fusion.** The fusion is situated in the chromosome at \textit{fim} in a \(\Delta lacZYA\) mutant of strain MG1655 (El-Labany et al., 2003). \(\beta\)-galactosidase assays were conducted as described by Miller (Miller, 1972), following growth of the wild type or \textit{dam} mutant strain in RD glycerol medium at 37°C with rapid aeration to an OD\textsubscript{600} of 0.2. Experiments were repeated at least twice, and the values shown represent the mean of at least four samples with 95% confidence intervals included for each value.

<table>
<thead>
<tr>
<th>Growth medium</th>
<th># ON microcolonies followed</th>
<th># ON to OFF events</th>
<th># OFF microcolonies followed</th>
<th># OFF to ON events</th>
<th>Final size (cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defined rich</td>
<td>2</td>
<td>7</td>
<td>26</td>
<td>24</td>
<td>250-500</td>
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<td>MOPS+succinate</td>
<td>35</td>
<td>19</td>
<td>400</td>
<td>119</td>
<td>32-128</td>
</tr>
</tbody>
</table>

**Table S1. Data Summary**

**References Supporting Information**


