Figure 1. Stochastic pulsing of flagellar promoters: (A) The flagellar operons are organized in a transcriptional three-tiered cascade and are accordingly labeled Class I (green), Class II (red) or Class III (blue). For simplicity, our diagram omits various branching and feedback regulatory pathways (such as the anti-sigma factor FlgM). (B) A microfluidic device (“mother machine”) constrains cells to grow in narrow linear tracks (SI). As the cells grow and divide, they are flushed away at the open end of the tracks by a constant flow of media, which ensures chemostatic growth. We monitor the cell confined at the bottom (the “mother cell”, red) to build a lineage over multiple cell divisions (SI). (C) Kymographs illustrating the pulsating dynamics of flagellar promoters. Each kymograph shows fluorescence (false-color, orange) from a strain harboring a YFP transcriptional fusion to a specific flagellar promoter from either Class I (top), II (middle) or III (bottom). Cells were grown in MOPS rich defined media (Methods) at 34°C and fluorescence images from each cell was acquired every 10 minutes. White dots indicate the location of the mother cell in each frame, which was identified via a constitutively expressed mCherry marker. Cells with the promoter encoding the Class I master regulator (top) are continuously fluorescent. By contrast, Class II (middle) and Class III promoters (bottom) stochastically switch between active and inactive transcriptional states over multiple divisions.
Figure 2. Promoters across classes show distinct dynamics but pulse simultaneously within their own class (II or III). (A) Typical activity of the sole Class I promoter, flhD, which controls the expression of the master regulator (green). Promoter activity is quantified by taking the cell-growth corrected time-derivative of the associated fluorescence signal (Methods). (B) (Top) Activity of fliF (dark red) and fliA (bright red) promoters within the same cell, representative of Class II pulsing dynamics. (Bottom) Correlation between two Class II gene reporters in the same cell as determined by flow cytometry (SI). Each strain harbors a reference reporter consisting of the fliF promoter and CFP and a second Class II promoter fused to YFP. The control promoter is a synthetic constitutive promoter. (C) Activity of fliC (dark blue) and motA (light blue) promoters within the same cell, representative of Class III pulsing dynamics. (Bottom) Correlation between two Class III gene reporters in the same cell as determined by flow cytometry. Similar to (B), each strain harbors a reference reporter consisting of the fliC promoter and CFP and a second Class III promoter fused to YFP. The control promoter is again a synthetic constitutive promoter. (D) Normalized autocorrelation function of flagellar promoter activity of Class I (green), II (red), and III (blue), estimated from the activity of flhD, fliA, and tar promoters, respectively (Methods). (E) Cumulative distribution of the pulse “on” durations, plotted as log(1-CDF). Shown are the distributions for fliF (Class II, red) and fliC (Class III, blue) (F) Cumulative distribution of the pulse “off” durations, plotted as log(1-CDF). Shown are the distributions for fliF (Class II, red) and fliC (Class III, blue).
Figure 3. Class II pulses do not require transcriptional or translational endogenous regulation of the master regulator. (A) Top, Endogenous expression of the master regulator FlhDC is driven by the class I promoter, whose regulation is controlled by multiple transcription factors (TF)\(^{(42)}\). In addition, translation of FlhDC is regulated by several small RNAs (sRNAs) interacting with the 5′ untranslated region (UTR) of the FlhDC transcript (top left). We replaced the native Class I promoter with a synthetic constitutive promoter (Pro4)—additionally, we altered the 5′ UTR so that the synthetic ribosomal binding site (RBS), which drives FlhDC translation is insensitive to sRNA regulation (top right). Bottom, typical Class II promoter dynamics (fliF promoter, red), wild-type (bottom left) and “constitutive” (bottom right) FlhDC promoters. (B) Cumulative distribution function (plotted as 1-CDF) of Class II promoter activity amplitudes from wild-type (solid red circle) and “constitutive” strains (open red circle). (C) Normalized autocorrelation function of Class II promoter activity, wild-type (dark red), synthetic “constitutive” (light red). (D) Simultaneous measurements of fluorescence signal from the Class I reporter (green) and activity of the Class II promoter fliFp (red) within the same cell. The fluorescence signal from the Class I reporter is a proxy for the concentration of proteins produced from the FlhDC promoter while the Class II promoter activity is the time derivative of the fluorescence signal from the Class II reporter which is a proxy for transcription from that promoter. Typical examples of wild-type (upper) and ΔYdiV cells (lower) along
with the Pearson correlation coefficient for the Class I and Class II signals (Methods). For ease of visualization, each signal is normalized so that the minimum value of the signal is 0 and maximum value of the signal is 1. Pearson correlation coefficient was computed on the raw data prior to normalization. 

(E) Normalized histogram showing distribution of correlation coefficients from 100 lineages for wild-type (blue) and for ΔYdIV (yellow) strains. Solid lines, kernel density approximations of those distributions (red and purple respectively). Each lineage is at least 30 generations long.

Figure 4. Modulation of Class III pulses by post-translational regulation of the alternative sigma factor, FliA. (A and B) Paired measurements of Class II and Class III within the same cells. We compared fluctuations in Class II fluorescence signal (red, fliFp), a proxy for the concentration of proteins produced from Class II promoters, to the activity of a Class III promoter (fliCp, blue). Typical examples, (A) wild-type cells, and (B) a ΔFlgM mutant that constitutively expresses the master regulator, FlhDC, to bypass any transcriptional feedback (SI). (C) Mean Class III activity as a function of the Class II reporter concentration in cells harboring fliF and fliC promoter reporters. Binned average of single cell measurements, wild-type (circles) and ΔFlgM (diamonds) strains; ΔFlgM mutant, same as in (B).
Figure 5. Model of flagellar regulation using a hysteretic switch (A and B) Input-output relationship between Class I and Class II. We plotted Class II (fliFp) promoter activity (Methods) as a function of a wide range of Class I levels using synthetic promoters (Pro1-5, ProB). For each strain associated with a different synthetic promoter, we divided Class I reporter levels into 5 logarithmically-spaced bins. From each bin we plotted the mean input (i.e. Class I) against the mean of the corresponding output (i.e. Class II). (A) ΔYdiV mutant strains, and (B) strains with wildtype YdiV levels. Error bars indicate standard deviation of Class II activity. In (A), dashed lines with arrows are guides for the eyes to delimit the hysteretic behavior in ΔYdiV mutants where Class II activity appears to switch abruptly. In (B), black arrows highlight two strains whose Class II activity shows the greatest difference with that of ΔYdiV mutants. (C) Hypothetical model of FlhDC regulation based on results in (A) and (B). The hysteresis in YdiV strains suggests the existence of positive-feedback loops (that may be direct or indirect) which resist change from inactive to active states or vice versa. YdiV, possibly due to its ability to disrupt the feedback, increases the probability of transitions between inactive and active states. We note that these interactions are likely to occur at the post-translational level since our synthetic promoters remove endogenous transcriptional and translational regulations of flhDC. (D) Commitment versus bet-hedging behavior in ΔYdiV and wildtype cells. Each strip is a typical kymograph of a strain harboring a Class II promoter reporter expressing FlhDC from synthetic promoters Pro1, Pro2, Pro4 or Pro5. For visual aid, a constitutive marker (gray) was merged with the Class II reporter fluorescence (orange/yellow).