Convergent transcription confers a bistable switch in Enterococcus faecalis conjugation

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Edited by Richard P. Novick, New York University School of Medicine, New York, NY, and approved April 19, 2011 (received for review January 30, 2011)

Convergent gene pairs with head-to-head configurations are widespread in both eukaryotic and prokaryotic genomes and are speculated to be involved in gene regulation. Here we present a unique mechanism of gene regulation due to convergent transcription from the antagonistic prgX/prgQ operon in Enterococcus faecalis controlling conjugal transfer of the antibiotic resistance plasmid pCF10 from donor cells to recipient cells. Using mathematical modeling and experimentation, we demonstrate that convergent transcription in the prgX/prgQ operon endows the system with the properties of a robust genetic switch through premature termination of elongating transcripts due to collisions between RNA polymerases (RNAPs) transcribing from opposite directions and antisense regulation between complementary counter-transcripts. Evidence is provided for the presence of truncated RNAs resulting from convergent transcription from both the promoters that are capable of sense–antisense interactions. A mathematical model predicts that both RNA collision and antisense regulation are essential for a robust bistable switch behavior in the control of conjugation initiation by prgX/prgQ operons. Moreover, given that convergent transcription is conserved across species, the mechanism of coupling RNA collision and antisense interaction is likely to have a significant regulatory role in gene expression.

Convergent transcription from two opposing promoters of partially overlapping genes on opposite strands of DNA gives rise to transcripts with potential sense–antisense interactions in the overlapping region. Such convergent transcription is widespread in eukaryotes including the mouse (1), human (2), Drosophila melanogaster (3), and Arabidopsis thaliana (4) genomes and a number of prokaryotes such as Listeria monocytogenes (5), Mycoplasma pneumoniae (6), and Escherichia coli (7). We postulate that convergent, overlapping gene organization provides two-layered regulation by a combination of transcriptional interference and antisense regulation through RNA:RNA interactions between complementary transcripts (8).

Transcription from promoters of convergent overlapping genes gives rise to a finite probability that opposing elongating RNA polymerases (RNAPs) collide head-on, thus exerting a suppressive effect on transcription. Such suppressive influence of one transcriptional activity on a second transcriptional activity occurring in cis is referred to as transcriptional interference (9), as has been reported in prokaryotic (10, 11) and eukaryotic systems (12, 13). Furthermore, the two transcripts, having complementary sequence in the overlapping region, may exert antisense regulation through RNA:RNA interactions (8, 14). Given the frequent occurrence of sense–antisense transcripts in both prokaryotic and eukaryotic genomes, convergent transcription may play a significant role in gene regulation by both mechanisms. However, there have been few attempts to document the relative effect of each mechanism in control of transcription of overlapping, convergent genes.

The present study investigates the role of transcriptional interference arising due to RNA collision and antisense regulation due to expression of complementary transcripts from operons prgQ and prgX, in plasmid pCF10 that regulates the conjugal transfer of antibiotic resistance between Enterococcus faecalis donor and recipient cells (15). Promoter P_{O} drives expression of the prgQ operon, which encodes pheromone inhibitor peptide, iCF10 (AITLIFI) as well as the pCF10 conjugation machinery (16). Approximately 223 bp downstream of the prgQ start site, the convergent promoter P_{X} drives expression of the prgX operon which encodes repressor PrgX regulating P_{Q} transcription by binding to the operators XBS 1 and 2 of promoter P_{Q} (Fig. 1A–C and SI A and B). In addition to the full-length prgX transcript (denoted X), transcription from P_{X} also produces a 104-nt non-coding RNA, Anti-Q, by processing or termination within the 5′ end of the prgX transcript (17). The interaction of Anti-Q with nascent prgQ transcripts affects folding of prgQ RNA and results in a short 380-nt terminated Q_{S} transcript (at IRS1, Fig. 1A) that encodes iCF10, but is incapable of inducing conjugation (18).

P_{Q} is normally in a repressed state whereas P_{X} is constitutive (Fig. 1B). The switch of P_{R} between the repressed state (conjugation off, Fig. 1B) and the derepressed state (conjugation on, Fig. 1C) is controlled by competing interactions of PrgX with inhibitor iCF10 and an inducer, cCF10, a chromosomally coded heptapeptide (LVTLLFV) that is produced predominantly by recipient cells (16) because in donor cells, the pCF10-encoded PrgV protein sequesters endogenous cCF10 (19, 20). In the absence of recipient cells and thus low ratios of cCF10 to iCF10, iCF10-bound PrgX tetramers cause a DNA loop between XBS 1 and 2 sites, stabilizing a repressing complex at P_{Q} (Fig. 1B) (21, 22). In the derepressed state, the cCF10 imported from the environment via membrane protein PrgZ (23) displaces iCF10, resulting in the disruption of PrgX-iCF10 tetramers and the DNA loop (Fig. 1C). This disruption leads to increased levels of prgQ transcription, which serves to titrate the Anti-Q RNA, and results in expression of a longer 530-nt transcript called Q_{L} (Fig. 1A), capable of inducing conjugation by a post-transcriptional mechanism (24).

In this report we used a mathematical model to demonstrate that convergent transcription of the prgX/prgQ system plays a regulatory role in endowing the system with a bistable switch-like behavior, a characteristic of many robust biological switches, including regulation of competence in Bacillus subtilis (25), lysozyme and lytic state in bacteriophage λ (26), and during cell fate determination in Xenopus laevis oocytes (27). We provide experimental evidence that RNAP collision gives rise to shorter truncated transcripts that exert an antisense effect on transcripts of prgX and prgQ. Both transcriptional interference as a result of

Author contributions: A.C. and W.-S.H. designed research; A.C. and C.-C.S. performed research; A.C., C.M.J., E.-C.S., Y.N.K., D.R., and G.M.D. contributed new reagents/analytic tools; A.C., C.M.J., G.M.D., and W.-S.H. analyzed data; and A.C., G.M.D., and W.-S.H. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1101569108/DCSupplemental.
A two-part mathematical model was formulated to describe the mechanism and to quantitatively evaluate the behavior of transcription from P0 and PX in response to inducer cCF10 (9). The first part is a discrete model that enumerates the frequency of RNAP collision and the generation of truncated RNA (Figs. S2 and S3). The second part is an ordinary differential equation (ODE)-based mathematical model on the mass balance of the components of the pCF10 system (Fig. 1D, Fig. S4, and Tables S1–S6).

The discrete model simulated RNAP moving along the overlapping DNA between P0 and PX (SI Text and Fig. S2). Upon RNAP binding to the promoter, a delay of 10 s is allowed for formation of the elongation complex (28) before RNAP moves at a velocity of elongation at 50 bp/s, which has been reported as theaverage velocity of RNAP in E. coli in the presence of pausing (29). The maximal RNAP binding rate at a derepressed P0 was set at 0.1/s on the basis of RNAP initiation rates estimated for the Pbla system in E. coli (30) and the PR-PRE system in bacteriophage λ (10). Using experimentally determined relative strengths of promoters P0 and PX under repressed and derepressed states in Fig. 2, the RNAP binding rates at P0 and PX under these two states were calculated. RNAP collision is assumed to occur when converging RNAPs from P0 and PX are separated by 60 bp (RNAP footprint). This result takes into consideration occlusion of P0 due to passage of RNAP originating from PX and vice versa. Our experimental data suggested that RNAP collision did not result in complete abolition of transcription on the basis of a 10% level of X transcript observed upon derepression; we thus assume that 90% of RNAP collisions result in transcriptional termination (31), releasing short transcripts terminated at the point of collision (SI Text and Fig. S2).

Movement of RNAP is not hindered by binding of PrgX to DNA (Fig. S5A). Successful passage of RNAP across the overlapping region (223 nt) without collision generates the full-length Q or X RNA. RNAP collision gives rise to a population of truncated RNA with varying length of <223 nt from P0 and PX (Fig. 1D). Previous work showed that stem loops at 46–51 nt and 80–83 nt of PX transcript respectively interact with stem loops at 173–178 nt and 156–161 nt of PX transcript (Fig. S3) (32). On the basis of RNAP collision and antisense regulation are necessary for the prgQ/prgX genetic switch to function in a robust manner.

Results
Modeling the prgQ–prgX Genetic Network: Coupled Effect of RNAP Collision and Antisense Regulation. A model for conjugation control by the prgX/prgQ operons is shown in Fig. 1D. The proposed model is based in part on previously described mechanisms (15, 18). We included additional regulatory effects contributed by transcriptional interference caused by RNAP collision and antisense effects caused by truncated RNA produced as a result of transcription interference. The underlying hypothesis is that RNAP collision causes failure in transcription, thus suppressing the transcription rate from both promoters. A second element is the generation of truncated transcripts that are released upon RNAP collision. We consider the hybridization of these short RNAs to their complementary counter-transcripts.

![Fig. 1. The prgQ–prgX genetic locus controls conjugation of pCF10 plasmid in Enterococcus faecalis. (A) Convergent promoters P0 (red) and PX (green) drive expression of the antagonistic prgQ and prgX operons, respectively, with a 223-bp overlap. Transcripts (indicated by thick arrows) from the inducible P0 promoter include 380 nt Q, RNA terminating at an inverted repeat sequence (IR51) and 530 nt Q RNA that results in expression of downstream conjugation-related genes. Transcripts from constitutive antisense PX promoter include noncoding Anti-Q and full-length X RNA coding for PrgX protein. (B and C) Promoter PX in the repressed state (B) and the derepressed state (C). (D) Model for the prgQ–prgX genetic switch. Successful transcription gives rise to full-length Q and X RNA (>223 nt). Failed transcription, upon collision of converging RNAP, gives rise to various sizes of truncated RNA (≤223 nt) indicated by dashed arrows. Some truncated RNAs (Q104 and X104–223nt) have the potential to exert antisense interaction with full-length counter-transcripts (not to scale).](image-url)

<table>
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<th>State</th>
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![Fig. 2. Interference exerted on P0 expression due to convergent transcription. (i) pCJ1 (OG1Sp), single PX promoter construct; (ii) pBK1-25 (OG1Sp), single PX promoter construct in derepressed (D) state; (iii) pBK1-25 (100-5), single PX promoter construct in repressed (R) state; (iv) pBK1 (OG1Sp), convergent promoter construct measuring expression from derepressed PX in the presence of PX; and (v) pBK1 (100-5), convergent promoter construct measuring expression from repressed PX in the presence of PX. The lacZ mRNA expression levels (normalized to gyrA<sup>−</sup>) shown are an average of three independent qPCR experiments.](image-url)
of these data and in silico RNA structural analysis using Sfold (33), we postulate that truncated P_X RNAs (including Anti-Q RNA) between 104 nt and 223 nt in length and truncated Q RNA between 180 nt and 223 nt are capable of RNA-RNA interaction with their counter-transcripts Q and X, respectively, due to the presence of stem loops (Fig. S3). These subpopulations of truncated RNAs capable of antisense effects are collectively denoted as Q_{AR} (180–223 nt) and X_{AR} (104–223 nt) RNA from P_O and P_X promoters, respectively (Fig. 1D).

From the discrete model simulation the rates of generation of Q, X, Q_{AR}, and X_{AR} species were calculated (Fig. 3.4 and B) for simulation using the ODE model (SI Text and Fig. S2). A set of eight ODEs describes the balance of RNA species Q, X, Q_{AR} and X_{AR}, intracellular iCF10 and cCF10, and extracellular iCF10 (Eqs. S25–S32), considering the rate of production, degradation, and dilution due to expansion volume caused by growth as well as interactions among these components (SI Text). For RNA species, interaction between sense transcripts (Q and Q_{AR}) and antisense transcripts (X and X_{AR}) following second-order kinetics (32) was also considered (SI Text). Hybrid RNA duplex between X RNA and Q_{AR} is considered to be unavailable for translation (Fig. 1D) (8). The formation of RNA duplex between X_{AR} and nascent Q RNA gives rise to formation of Q_{2} RNA (18, 32). Nascent Q transcripts that do not terminate proceed to elongate and form Q_{L}. Translocation of Q_{S} and Q_{L} leads to production of the secreted inhibitor peptide iCF10 (15). Transport of iCF10 and cCF10 across the membrane protein PrgZ is considered to follow first-order kinetics (23). The model assumes that due to cCF10-sequestering activity of PrgY protein, the effect of endogenous cCF10 in donor cells is negligible (20).

The ODE model was solved to obtain a steady-state solution to a fixed concentration of extracellular cCF10 (Fig. 3 C and D). The parameter values were obtained from the literature or experimental data (Tables S3 and S4). To test the sensitivity of the steady-state behavior to the parameter values, the parameters in the model were lumped into six dimensionless parameters (Tables S3 and S4) to reduce the model to a set of six non-dimensional ODEs (Eqs. S48–S53). The values of three of these dimensionless numbers were determined from experimental data (SI Text), whereas the remaining three were obtained from literature and subjected to sensitivity analysis (Table S6). In the dimensionless model, the term corresponding to RNA interaction between truncated RNAs X_{AR} and Q_{AR} was eliminated as it did not affect the steady-state behavior of the system. Simultaneous parameter space search for the remaining three dimensionless parameters demonstrated that steady-state behavior shown in Fig. 3 C and D existed over at least two orders of magnitude (Fig. S4A and Table S6). The details of model derivation are described in SI Text.

P_O Activity in Presence and Absence of Convergent Transcription. The strength of P_O in the presence (repressed state) and absence (derepressed state) of repressing PrgX complexes was quantified by measuring steady-state expression levels of the lacZ transcript from plasmids containing either a single P_O promoter or both convergent promoters with the lacZ reporter fused downstream of IRS1 (Fig. 2). Two kinds of host strains were used: (i) OG15P, a wild-type host strain; and (ii) 100-5, a derivative of OG15P that provides PrgX in trans from the chromosome at levels similar to wild-type pCF10 plasmid (Fig. S1B). By combining the plasmids and host strains a set of five conditions was created as shown in Fig. 2. The intrinsic promoter strength of P_O (derepressed state) in the absence of convergent transcription is 8.3-fold higher than that of the constitutive P_X (Fig. 2, ii vs. i). The repression of P_O by supplying PrgX in trans caused a 9-fold reduction in expression in the absence of convergent transcription (Fig. 2, iii vs. ii). In the repressed state, P_O has almost identical strength to P_X (Fig. 2, i vs. iii). In the derepressed state P_O becomes the stronger promoter (Fig. 2, ii vs. iv). Whereas convergent transcription from P_X caused only a modest (~15%) reduction of transcripts from P_O in the derepressed state (stronger P_O than P_X; Fig. 2, ii vs. iv), it reduced P_O transcript levels by ~90-fold in the repressed state (similar strength of P_O and P_X; Fig. 2, ii vs. v). These results show that convergent transcription greatly enhances the effect of repression on P_O.

Model Simulation and Bistable Behavior. The discrete model was used to predict the relative abundance of transcripts under repressed and derepressed conditions as a result of RNAP collision (Fig. 3 A and B and Tables S1 and S2). Under the repressed condition, a higher number of transcription events are predicted to give rise to full-length X RNA, compared with the depressed condition, whereas the opposite is predicted for full-length Q RNA. On the other hand, a broader distribution of Q_{AR} and X_{AR} RNA is predicted in the repressed state compared with the derepressed state. In the derepressed state, the 8.3-fold stronger P_O not only increases Q transcription but also knocks off most RNAP initiated from P_X at loci proximal to the P_X promoter. As a result, RNAP collision results in only a moderate decrease in Q transcription, but a rather large decrease in X transcription.

Using the ODE model we next examined the effect of RNAP collision and antisense regulation on the steady-state level of Q_{L} in response to the signaling molecule cCF10. The level of Q_{L} RNA is an indicator of the state of conjugation, because it encodes genes of conjugation machinery. Steady-state solution of the model shown in Fig. 3C demonstrates a characteristic bistable response of Q_{L} to cCF10 concentration. Multiple steady states reside in the S-shaped section of the curve. Two are stable steady states corresponding to on (upper) and off (lower) states, respectively. The unstable steady state in the middle is not observed experimentally. For the system at an off state, as cCF10 concentration increases it moves along the lower stable steady state (Q_{L} < 1.4 nM) until cCF10 reaches 3.7 ng/mL, and then the system undergoes a sharp transition to an on state (conjugationally competent) corresponding to a high level of Q_{L} (>3.8 nM). Conversely if the system is initially at an on state, it remains at an on state till cCF10 concentration decreases below 3.2 ng/mL where it rapidly changes to an off state. The system is thus marked by well-separated (c(cCF10 > 3.7 ng/mL) and off states (cCF10 < 3.2 ng/mL).
We evaluated the relative contribution of RNAP collision and antisense regulation to bistable behavior by eliminating RNAP collision (RC) or antisense regulation (AR) or both from the model (Fig. 3D). AR was eliminated from the model by setting the rate constant for RNA interaction to zero, whereas RC was eliminated by setting the transcription rates of O and X RNA to RNAP firing rates from P_O and P_X, respectively, and X_{AR} and Q_{AR} concentration to zero (Table S5). Bistability is observed only when both RC and AR are present (RC/AR). When AR is absent and RC is present (RC/AR^-) or both RC and AR (RC^-/AR^-) are eliminated, no bistability is seen (Table S6). We also examined the case where RC is only partially removed by reducing the RNAP falling-off rate upon RNAP collision. As the falling-off rate decreases to 50%, the steady-state behavior transitions from a bistable to a ramp-like response (Fig. S4B). The results thus hinted that the 104-nt Anti-Q RNA, produced as a result of X RNA processing, alone is insufficient for the bistable behavior. This result is indeed the case for RC^-/Anti-Q, where the bistable response to cCF10 is lost and a ramp-like transition is observed (Fig. 3D).

Inverse Expression and Response to Pheromone. We next measured the levels of transcripts from P_X and P_P promoters in strain OG1Sp carrying wild-type (WT) plasmid pCF10. Under repressed conditions the Q1 transcript level was much lower than the X transcript level even though the strengths of P_O and P_P are similar. Upon induction with high levels of cCF10 (50 ng/mL), the Q1 transcript level increased >200-fold, whereas the X transcript decreased ~9-fold (Fig. 4A). The opposite trend in their response to derepression is consistent with model predictions (Fig. 3C and Fig. S4C). The level of Q_L RNA observed was lower than that seen in the lacZ reporter system (Fig. 2). This result could be attributed to a high degradation rate for Q_L. This notion is consistent with model simulation, where a 10-fold decrease in Q_L degradation rate abolishes bistable behavior (Fig. S4D).

A dose/response curve of Q_L transcript to cCF10 showed that Q_L expression remains low below 0.5 ng/mL of cCF10, followed by an 8.3-fold increase at 5 ng/mL of cCF10 (Fig. 4B and Fig. S4E), characteristic of a switch from the off to the on state as predicted by the model (Fig. 3C). Flow cytometric data using GFP protein expression as an indicator of on state confirm that the system transitions from an off to an on state between 0.5 and 5 ng/mL (Fig. S4F).

Truncated RNA from P_O and P_P. To verify the existence of the truncated transcripts (X_{AR} and Q_{AR}) in pCF10 carrying donor (OG1Sp) cells, we used Northern blots with sets of probes complementary to the 5’ (Q1 and X1) and 3’ segments (Q2 and X2) within the overlapping region of the respective RNAs from each promoter (Fig. 4C, Upper). Shorter (truncated) P_O (Fig. 4C, lanes 1–4) and P_P (Fig. 4C, lanes 5–8) transcripts within the overlapping region were indeed observed under both repressed and derepressed conditions. The Q1 probe detected truncated transcripts of ~100-, 150-, and 200-nt sizes, with higher relative abundance in repressed cells (Fig. 4C, lane 2 vs. lane 1, and Fig. S5C). Similarly, the X1 probe detected truncated RNAs in the size range between 80 and 200 nt (Fig. 4C, lanes 5 and 6). The prominent band of ~100 nt corresponds to the 104 nt Anti-Q RNA reported previously (17). The levels of truncated transcripts detected by probes Q1 and X1 (Fig. 4C, lanes 1 and 2 and 5 and 6) proximal to the 5’ end are higher than those detected by distal probes Q2 and X2 (Fig. 4C, lanes 3 and 4 and 7 and 8), consistent with the higher collision frequency near the 5’ end. The presence of a short 100-nt band detected by the X2 probe (Fig. 4C, lane 8) indicates an additional mechanism involved in processing of longer P_X transcripts. However, this truncated transcript is unlikely to have a regulatory role in the switch behavior, due to the absence of stem loops I and II required for antisense interaction with P_P transcripts.

The high abundance of truncated RNA in the repressed state (Fig. 4C, lane 2 vs. 1 and lane 6 vs. 5, and Fig. S5C) is consistent with the prediction of the RNAP collision model (Fig. 3 A and B). The size of shorter RNAs observed falls within the range of Q_{AR} and X_{AR} considered in the model. However, it is possible that these short RNAs arise from degradation of full-length transcripts. In this case, the ratio of truncated RNA to full-length RNA would be similar under derepressed and repressed conditions. Conversely if these RNAs are the products of RNAP collision, we expect this ratio to increase for P_P transcripts in the derepressed state as X RNA decreases, whereas the ratio corresponding to P_O transcripts should decrease because of a dramatic increase in Q (sum of Q_s + Q_h) transcripts upon induction. Quantification of Northerns in Fig. 4C (lanes 1 and 2 and 5 and 6) indeed shows that the ratio of truncated to full-length RNA increases for P_X transcripts and decreases for P_O transcripts in the derepressed state compared with the repressed state (Fig. 4D).

Decoupling RNAP Collision and Antisense Regulation. The relative contribution of RNAP collision and antisense regulation to switch response was evaluated by decoupling these effects using single or convergent promoter constructs in host strains OG1Sp and 100-5 for repressed and repressed conditions, respectively (Fig. 5A). Three cases were analyzed: convergent transcription (RC/AR), no RNAP collision in absence of antisense RNA (RC^-/AR^-), and presence of antisense RNA supplied in trans (RC^-/AR) using plasmid constructs shown in Fig. 5A. Transcription from P_P in the presence of convergent transcription (RC/AR) showed abundant truncated RNA from a repressed P_O compared with a derepressed P_O (Fig. 5B, lane 2 vs. lane 1). The longer (>700 nt), likely X transcript, was more abundant in the repressed condition, consistent with our model pre-

**Fig. 4.** Inverse relationship of Q_L and X expression and presence of truncated RNAs in the prgQ-prgX locus. (A) Q_L and X RNA levels measured by qPCR using X-specific and Q_L-specific primers from OG1Sp (pCF10) under repressed and derepressed conditions. (B) Levels of Q_L RNA in response to different concentrations of cCF10. Data shown in A and B are averages of three independent experiments (error bars are SDs from mean values). (C) (Upper) Map for the single-stranded RNA probes Q1, Q2, X1 and X2 used for Northern blotting experiments. (Lower) Northern blots to detect transcripts from P_O and P_P in cells carrying WT pCF10, in either the derepressed (D) or the repressed (R) state. Probes and lanes: Q1, lanes 1 and 2; Q2, lanes 3 and 4; X1, lanes 5 and 6; and X2, lanes 7 and 8. (D) Quantitative estimation of intensity of bands for truncated P_O and P_P transcripts in lanes 1 and 2 and 5 and 6 normalized to intensity of Q_s + Q_h and X, respectively, using ImageJ software (National Institutes of Health).
Fig. 5. Decoupling the effects of RNAP collision (RC) and antisense regulation (AR) in the prgQ−prgX locus. (A) Plasmid constructs containing either Pr (red) or Pvas (green) or both promoters used to decouple RC and AR effects (lanes indicated). Derepressed vs. repressed states are compared using strains OG15p (derepressed) vs. 100-5 (repressed). Antisense interactions in the absence of RC were assessed by supplying complementary RNAs in trans using the plasmids diagrammed on the right. (B) Upper) Transcripts from Pr probed with X1. (Lower) Transcripts from Pvas probed with Q1. Lanes and corresponding constructs/host strains used are as indicated in A.

Discussion

Our results demonstrate that a head-to-head convergent promoter system can accomplish two levels of regulation: (i) enhancing the degree of repression through RNAP collision and (ii) suppressing transcript levels through interactions with cis-encoded antisense RNA produced from the overlapping region. With RNAP collision alone, an 8.3-fold increase in Po strength is predicted to give rise to a 12-fold increase in successful transcription from Po (Fig. 3A) relative to the repressed state. Experimentally, a much greater difference in Q transcripts was observed using both a reporter system (Fig. 2) and direct measurement of Q transcripts (Fig. 4A). This enhanced difference is accomplished by a greater degree of suppression of Q transcript level under the repressed conditions through the products of RNAP collision, namely truncated RNAs that have an antisense regulatory effect on transcripts from both Po and Pvas. Using a combination of host strains and constructs to mimic conditions of RC and AR, we show that Q transcripts that were suppressed under repressed conditions with RC/AR became elevated when RC or both RC and AR were eliminated (Fig. 5B). Providing constant levels of Anti-Q RNA did not restore switch behavior, as demonstrated both experimentally (Fig. 5B, lane 8) and theoretically (Fig. 3D). The sharp difference in the level of Anti-Q RNA and Q species between the repressed and the derepressed state due to RC and AR contributes to the switch behavior.

Both mathematical modeling (Fig. 3A and B) and experimental observations (Fig. 4C) indicate the presence of certain prominent sizes of truncated RNA. However, the stochasticity of RNAP firing from promoters is expected to blur the discrete nature of truncated RNAs, pointing toward the presence of potential RNAP pause sites within overlapping DNA, thus increasing the probability of collision at those sites. Such an effect will further strengthen the bistability due to enhanced effects of RNAP collision and antisense regulation (10).

Many biological switches have been shown to exhibit bistability in steady-state analysis of their system models. The pivotal role of RC and AR in achieving bistability is evident from our work. Because the donor cells must turn their conjugation system on only in response to true signal, a switch type of regulatory mechanism allows for a tighter control of conjugation.

repression, although RNAP collision plays a more dominating role as predicted by the model (Fig. 3B). The presence of shorter RNA (100–223 nt) in lane 4 compared with lane 3 in Fig. 5B suggests that these are products of endonucleolytic cleavage within paired regions formed by the “kissing” interactions between the loop structures of QAR and Q transcripts (Fig. S3) (32).

Similarly, during convergent transcription QL was expressed at higher levels in the depressed state than in the repressed state (Fig. 5B, lane 5 vs. lane 6), whereas higher abundance of truncated RNA was seen under the repressed state (Fig. 5B, lane 6). In the absence of RNAP collision and antisense regulation, longer QL and QN RNAs were expressed from Pvas even under repressed conditions (Fig. 5B, lane 7). The lack of truncated RNA in this case in the absence of RNAP collision (Fig. 5B, lane 7) is consistent with our model that these RNAs are mainly a result of convergent transcription. When we provided excess amounts of antisense 104-nt Anti-Q RNA, representing XAR species in the model, in trans from a high copy number plasmid (Fig. 5B, lane 8), QL expression decreased (Fig. 5B, lane 7 vs. lane 8); however, it was visible at lower exposures. Importantly, QN RNA was expressed in RC+/AR− and RC+/AR cases even under repressed conditions. Intensity of bands corresponding to both X (Fig. 5B, lanes 1–4) and QN RNA in (Fig. 5B, lanes 5–8) normalized to 5s RNA shows that the high degree of suppression of X in the derepressed state (Fig. 5B, lane 1) and QL in the repressed state (Fig. 5B, lane 6) occurs only during convergent transcription (Fig. S5D). These results are consistent with model prediction.

The absence of convergent transcription (RC+/AR−) resulted in increased expression of both Anti-Q RNA (~100-nt band) and longer X transcripts (Fig. 5B, lane 3), compared with the case with convergent transcription (lane 1), consistent with the negative effects of RNAP collision and antisense regulation on Pvas expression. A 200-nt band observed in the RC+/AR− case was not observed in the RC/AR case. This result could be caused by the presence of intrinsic pause sites, which were identified during in vitro transcription assays using conditions of RC+/AR− (Fig. S5B). It is possible that in the RC/AR case, converging RNAP from Pvas knocks off elongating RNAP from Pvas before reaching this locus.

The antisense effect (RC+/AR−) was restored (Fig. 5B lane 4) by providing excess amounts of shorter 253-nt RNA from Pvas in trans from a high copy number plasmid to the construct used in lane 3. The 253-nt RNA species contained the sequence of the overlapping region and was used to represent QAR species in the mathematical model. Compared with the RC−/AR− case (Fig. 5B, lane 3), providing excess antisense QAR RNA reduced the expression of X and Anti-Q transcripts significantly. However, the extent of suppression of X (~700 nt) was lower than that observed in the RC/AR case, as the X transcript was still present even when large amounts of QAR RNA were supplied (Fig. S3D). Comparison of the intensity of X transcripts normalized to 5s RNA shown in lanes 3 (~0.9) and 4 (~0.3) (Fig. S5D) indicates that RNAP collision is responsible for two-thirds of the decrease of X transcription in the derepressed state, exerting a stronger effect on X transcription than antisense regulation. These results suggest that both RNAP collision and antisense regulation are required to achieve high degree of suppression of the X transcript upon derepression, although RNAP collision plays a more dominating role as predicted by the model (Fig. 3B).
Transcription interference has been reported to exert a depressive effect on transcription from the weaker promoter in rprpL and PR-PRE promoter pairs of bacteriophages 186 and λ, respectively (10, 11). In these studies, RNAP collision was the major contributor to the observed interference whereas antisense effects were concluded to be insignificant. Convergent transcription in the JME-4 locus of *Saccharomyces cerevisiae* was shown to result in antisense and sense transcripts that were inversely related between diploid and haploid cell types (36). Again, in this case, the inverse relationship was ascribed to transcriptional interference but not to antisense regulation. Other convergent transcription systems have also been reported to have inverse transcription levels under different conditions, including the *ecit* gene involved in mouse stem cell development (37) and the *mogR* locus of *L. monocytogenes* controlling cell motility (61). In a study of transcription of the *Pot2* locus in rice blast fungus, truncated transcripts were observed that led to the identification of an antisense promoter (38). In silico analysis on the mouse genome and a follow-up study using an oligo-microarray revealed nearly 2,000 sense–antisense transcripts (39). The presence of multiple-sized transcripts without poly(A) appeared to hint at the presence of truncated transcripts.

The fact that convergent transcription is ubiquitous and has persisted in evolution (40) is perhaps an indication that such gene organizations confer fundamental mechanisms of gene regulation.

We show that using a single repressor, convergent transcription allows for simultaneous regulation of two genes, giving rise to a complex, tightly regulated switch behavior. Critical to conferring the sophisticated control is the combined use of RNAP collision and antisense regulation. The collision frequency and antisense levels of transcription suppression are highly dependent on many factors, including relative promoter strength, the length of overlap, and the exact sequence that causes RNAP pausing and interaction. With such a wide range of possible outcomes, using subtle structural tuning, convergent transcription may be highly adaptable to become a robust controller for many complex cellular events.

**Materials and Methods**

Details for strains, reporter constructs, medium, and growth conditions are provided in SI Text. Details for Northern blotting and qPCR are provided in SI Text. The numerical solution to stiff differential equations for steady-state analysis was obtained using the MATLAB function solve. For scanning parameter space, the MATLAB function polyxpoly was used for checking multiple steady states.

**ACKNOWLEDGMENTS.** We thank Dawn Manias for conducting the Western blot for PrgX, Laura C. C. Cook for flow cytometric data, and Sarika Mehra and Anupam Charniya for helpful discussions. This work was supported by National Institutes of Health Grants GM081888 (to W.-S.H.) and GM049530 (to G.M.D.).