

# DNA looping provides stability and robustness to the bacteriophage $\lambda$ switch

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The bistable gene regulatory switch controlling the transition from lysogeny to lysis in bacteriophage  $\lambda$  presents a unique challenge to quantitative modeling. Despite extensive characterization of this regulatory network, the origin of the extreme stability of the lysogenic state remains unclear. We have constructed a stochastic model for this switch. Using Forward Flux Sampling simulations, we show that this model predicts an extremely low rate of spontaneous prophage induction in a *recA* mutant, in agreement with experimental observations. In our model, the DNA loop formed by octamerization of CI bound to the  $O_L$  and  $O_R$  operator regions is crucial for stability, allowing the lysogenic state to remain stable even when a large fraction of the total CI is depleted by nonspecific binding to genomic DNA. DNA looping also ensures that the switch is robust to mutations in the order of the  $O_R$  binding sites. Our results suggest that DNA looping can provide a mechanism to maintain a stable lysogenic state in the face of a range of challenges including noisy gene expression, nonspecific DNA binding, and operator site mutations.

biochemical networks | genetic switches | rare events | stochastic modeling | nonspecific DNA binding

The bistable developmental switch controlling the transition from lysogeny to lysis in bacteriophage  $\lambda$  is one of the best characterized gene regulatory networks (1). In the lysogenic state, the phage  $\lambda$  genome is integrated into the chromosome of the *Escherichia coli* host cell and is essentially dormant because of expression of the *cI* repressor gene, the product of which represses *cro* and other genes (Fig. 1). A transition to the lytic switch state can occur in response to DNA damage (via UV irradiation), when CI molecules are cleaved by RecA. Transcription of the *cro* gene from  $P_R$  then triggers a cascade of gene activation, leading to phage excision, replication, and cell lysis. In mutants where this cascade is blocked, a state with elevated Cro levels is stable for several cell generations. This is known as the anti-immune state (2). A simple and intuitive explanation has been presented for this bistability (1): in the lysogenic state, CI is dominant and *cro* is repressed; yet, once *cro* begins to be expressed, Cro dimers repress transcription of *cI*, making the transition to lysis inevitable. However, quantitative measurements have revealed a puzzle: the lysogenic state of the phage  $\lambda$  switch is both extremely stable (3–5) and robust to rewiring of its transcriptional regulatory interactions (3). These features have not yet been explained by mathematical models. Here, we present dynamical simulations of a stochastic mathematical model that reproduces this seemingly mysterious behavior. Our simulations provide evidence that DNA looping plays a key role in ensuring the stability and robustness of the switch. The molecular interactions controlling the transcription of *cI* and *cro* have been studied in great detail. CI and Cro bind as dimers to the operator sites  $O_{R1}$ ,  $O_{R2}$ , and  $O_{R3}$  (Fig. 1), which control expression of the *cI* and *cro* genes from the  $P_{RM}$  and  $P_R$  promoters. Transcription from the unactivated  $P_{RM}$  promoter is  $\approx 10$  times weaker than from  $P_R$ ; however, when a CI dimer is bound at  $O_{R2}$ , transcription from  $P_{RM}$  is enhanced to approximately the same level as from  $P_R$  (i.e., the 2 promoters can compete with one another only when CI is bound at

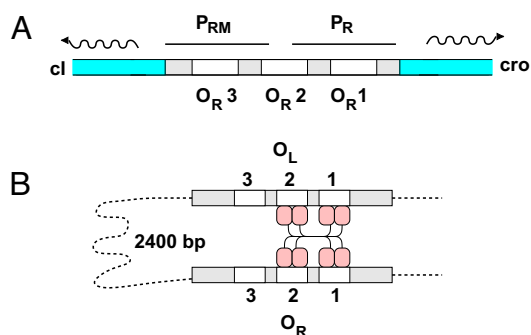


Fig. 1. Schematic illustration of the  $O_R$  region of the phage  $\lambda$  switch (A) and one of the possible DNA loops formed by octamerization of CI dimers bound at  $O_R$  and  $O_L$  (B).

$O_{R2}$ ). CI dimers bind preferentially and cooperatively to the  $O_{R1}$  and  $O_{R2}$  sites, which overlap the  $P_R$  promoter. When CI is bound to these 2 sites, *cro* ( $P_R$ ) is repressed and *cI* ( $P_{RM}$ ) is activated. Cro dimers bind preferentially to  $O_{R3}$ , which overlaps  $P_{RM}$ , so that when this site is occupied, *cI* is repressed (1). An important additional component of the network architecture is a DNA loop that can form between the  $O_R$  site and the left operator site  $O_L$ , located 2,400 bp from  $O_R$ , which also has 3 adjacent binding sites for CI dimers ( $O_{L1}$ ,  $O_{L2}$ , and  $O_{L3}$ ). This loop is mediated by octamerization between pairs of CI dimers bound at  $O_R$  and  $O_L$  (6). The role of this DNA looping interaction in the function of the phage  $\lambda$  switch remains a subject of debate (7–11).

Quantitative measurements have revealed several intriguing features of the phage  $\lambda$  switch. First, the lysogenic state is extremely stable (3–5), despite the stochastic nature of the underlying gene regulatory network. Stochastic fluctuations in gene regulation (“noise”) might be expected to cause spontaneous transitions from the lysogenic to lytic states, even in lysogens lacking RecA; yet the rate of these transitions is so low that it is almost unmeasurable. Second, recent measurements of  $P_{RM}$  and  $P_R$  promoter activity suggest that only a small fraction of the total CI in the cell is available for binding to  $O_R$  (7, 8, 12–14), whereas other measurements show that the total concentration of CI in the lysogen varies dramatically from cell to cell (15). Taken together, these results suggest that the stability of the lysogen is rather insensitive to the number of free intracellular CI molecules. Despite this stability,

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transition to lysis occurs readily in wild-type phage on UV irradiation, which leads to cleavage of CI by RecA. Finally, and remarkably, switch function is robust to changes in the gene network architecture itself: when the order of the 3  $O_R$  binding sites is altered so that  $O_{R1}$  is replaced by  $O_{R3}$  or vice versa, the network remains functional (3).

Computer simulations should be an excellent tool for explaining this behavior. Although stochastic simulations have successfully been used to model the initial developmental choice between lysogeny and lysis for lambda-infected cells (16), modeling spontaneous switching of an already established lysogen has proved problematic. Despite the fact that a wealth of biochemical parameters is available for this network, no model has yet reproduced its extraordinary stability and functional robustness (4, 12, 17–21). In this article, we present a model that takes into account the stochastic character of the chemical reactions and includes DNA looping and depletion of free CI and Cro by nonspecific binding to genomic DNA. Our model also explicitly describes the detailed dynamics of the binding of transcription factors to the promoters. Accurate computation of spontaneous switching rates for this large reaction set is achieved using the Forward Flux Sampling (FFS) rare event simulation method (22, 23), in combination with temporal coarse graining of dimerization and nonspecific DNA binding reactions. Our simulations show that this stochastic model can reproduce the bistability of the switch, its robustness to operator site mutations, and the extreme stability of the lysogenic state, even in the presence of nonspecific DNA binding.

In this work, we study the effect on switch function of 2 key parameters: the strengths of the DNA looping and nonspecific binding interactions. We find that the DNA looping interaction plays a crucial role. In the absence of the looping interaction, a highly stable lysogenic state can be achieved, but this state is very sensitive to depletion of free CI and to operator site mutations. When looping is included in the model, the lysogen is insensitive to CI depletion and robust to rearrangement of the operator sites. We conclude that DNA looping may play an important role in allowing the phage  $\lambda$  switch to function reliably even under highly destabilizing conditions in the host cell.

## The Model

Our model consists of a set of chemical reactions, simulated using the Gillespie algorithm (24). The components of the model are: dimerization of CI and Cro proteins, binding of CI and Cro dimers to specific DNA binding sites  $O_{R1}$ ,  $O_{R2}$ ,  $O_{R3}$ ,  $O_{L1}$ ,  $O_{L2}$ , and  $O_{L3}$ , binding of RNA polymerase (RNAP) to promoters  $P_{RM}$ ,  $P_R$ , and  $P_L$ , transcription of *cI* and *cro*, translation of the corresponding mRNA transcripts, degradation of mRNA transcripts, and removal of CI and Cro monomers and dimers from the cell. Our model also includes formation of a DNA loop between  $O_R$  and  $O_L$ , mediated by a CI octamer, and nonspecific binding of CI and Cro dimers to genomic DNA. The key parameters that we vary are the strength of the nonspecific DNA binding interaction  $\Delta G_{NSB}$  and the strength of the DNA looping interaction  $\Delta G_{loop}$ . Other parameters are fixed using biochemical data as far as possible. The model parameters are discussed in brief here and are described in full in the [supporting information \(SI\) Text](#).

**Host Cell Parameters.** We assume that the *E. coli* host cell is growing rapidly [doubling time, 34 min (3)], and has 3 copies of each of the  $O_R$  and  $O_L$  operators (4), in a cell volume of  $2 \mu\text{m}^3$  (4). The concentration of free RNAP in the cytoplasm is taken to be 50 nM (25), but our conclusions are not sensitive to this parameter, as we demonstrate in the [SI](#).

**Operator Binding Dynamics.** Equilibrium constants from the literature were used for CI (26, 27) and Cro (28) dimer binding to  $O_{R1}$ ,  $O_{R2}$ ,  $O_{R3}$ , for RNAP binding to  $P_{RM}$  and  $P_R$  (29) and for CI binding

to the  $O_L$  sites (8). Cro is assumed to bind to  $O_L$  and  $O_R$  sites identically. The total number of possible (unlooped) configurations of the  $O_R$  and  $O_L$  operators are, respectively, 40 and 36. Because we are performing dynamical simulations, we require rate constants  $k_a$  and  $k_d$  for association and dissociation. For all association rates, we used the diffusion-limited value  $k_a = 4\pi D\sigma = 0.314 \mu\text{m}^3 \text{s}^{-1}$  (taking the diffusion constant  $D = 5 \mu\text{m}^2 \text{s}^{-1}$  and the molecular size  $\sigma = 5 \text{ nm}$ ). The rate constant for dissociation, in  $\text{s}^{-1}$ , was then deduced from the equilibrium constant, using  $k_a/k_d = (\exp[-\Delta G/RT]) / (6.023 \times 10^{23} \mu\text{m}^3)$ , where  $k_a$  is in  $\mu\text{m}^3 \text{s}^{-1}$ ,  $\Delta G$  is in kcal/mol,  $RT = 0.616 \text{ kcal/mol}$  at  $37^\circ\text{C}$ , and  $6.023 \times 10^{23} \mu\text{m}^3$  is a volume conversion factor.

**Protein and mRNA Production and Removal.** We model transcription as a single reaction in which an mRNA molecule is produced when RNAP is bound to a promoter.  $P_{RM}$  activity is enhanced when a CI dimer is bound at  $O_{R2}$ . Transcription rates are  $0.014 \text{ s}^{-1}$  for  $P_R$ ,  $0.001 \text{ s}^{-1}$  for unstimulated  $P_{RM}$ , and  $0.011 \text{ s}^{-1}$  for stimulated  $P_{RM}$  (29–32). All mRNA transcripts are degraded with a half-life of 2 min. Translation and protein folding are combined into a single step. The model generates a statistical distribution for the number of proteins produced per transcript, which is governed by the balance between the translation and mRNA degradation rates. The average of this distribution (the “burst size”) is 6 and 20 for CI and Cro, respectively. The burst size for Cro follows ref. 4. The value for CI is based on the observation that the CI ribosome binding site (RBS) is  $\approx 7$ -fold weaker than that of LacZ (I. Dodd, personal communication), and that the LacZ burst size is 30–40 (33, 34). A somewhat weaker CI RBS was observed by Shean and Gottesman (35). Protein monomers and dimers are removed with rate constant  $\ln(2)/T_c$ , where the cell cycle time  $T_c = 34 \text{ min}$  (3). We also include active degradation of Cro monomers with half-life of 42 min (4, 13).

**Dimerization.** Dimerization free energies are taken to be  $-11 \text{ kcal/mol}$  for CI (36, 37) and  $-8.7 \text{ kcal/mol}$  for Cro (38). The association reaction is again assumed to be diffusion-limited. To increase the efficiency of our simulations, we coarse-grain the monomer–monomer association and dissociation reactions for both CI and Cro (39), as described in the [SI](#).

**DNA Looping.** When an  $O_R$  and an  $O_L$  operator each carry at least 2 adjacent CI dimers (at the 1–2 or 2–3 sites), these operators can associate to form a “looped state,” with rate  $k_{loop}$ , which dissociates with rate  $k_{unloop}$ . The total number of possible looped states is 49. Binding of CI dimers to the 2 nonoctamerized sites in a loop occurs with a cooperativity factor  $\exp(-\Delta G_{tet}/RT)$ , where  $\Delta G_{tet} = -3 \text{ kcal/mol}$  (8). Because we assume fast growth of the host cell, our model contains 3 copies of the host genome and 3 copies of the phage  $\lambda$  switch. Because the loop is much longer than the persistence length of DNA, we assume that any  $O_R$ – $O_L$  combination can form a loop. The strength and dynamics of the DNA loop in vivo are unknown. We therefore test the effects of DNA looping on the network behavior, by varying the ratio  $k_{loop}/k_{unloop} = \exp(-\Delta G_{loop}/RT)$ . We generally assume a fixed value  $62.1 \text{ s}^{-1}$  for  $k_{loop}$  (arising from considerations of polymer dynamics as discussed in the [SI](#)), but we find that only the ratio is important.

**Nonspecific DNA Binding Dynamics.** We model nonspecific DNA binding (8, 14) by including in our reaction set association and dissociation of CI and Cro dimers to  $10^7$  genomic DNA sites, corresponding to 2–3 copies of the bacterial genome. The association rate  $k_a$  is assumed to be diffusion-limited, and we assume identical nonspecific binding affinities for CI and Cro. Nonspecifically bound dimers are removed from the cell with rate constant  $\ln(2)/T_c$ . We do not model nonspecific binding of RNAP, because our value of 50 nM corresponds to the free RNAP concentration (25). To investigate the effects of nonspecific DNA binding on the model switch, we vary the parameter  $\Delta G_{NSB}$  where  $k_a/k_d =$



**Table 1. Spontaneous switching times (inverse of calculated switching rates) from the lysogenic to anti-immune states and from the anti-immune to lysogenic states, for wild-type phage  $\lambda$ , computed using FFS**

$\Delta G_{loop}$ , kcal/mol	$\Delta G_{NSB}$ , kcal/mol	Switching time lysogen $\rightarrow$ anti-immune (generations)	Switching time anti-immune $\rightarrow$ lysogen (generations)
No loop	No NSB	$(3.6 \pm 0.1) \times 10^9$	$2,300 \pm 100$
No loop	-2.8	$1,700 \pm 50$	$(7 \pm 1) \times 10^9$
-5.2	-4.1	$< 10^{23}$	$43 \pm 1$
-3.7	-2.8	$(3 \pm 2) \times 10^{25}$	$26 \pm 1$
-1.0	No NSB	$(6 \pm 2) \times 10^{14}$	$130 \pm 10$

A cell generation time of 34 min is assumed. FFS calculations used 8–85 interfaces, 1,000–10,000 configurations at the first interface, and 500–10,000 trials per interface, and were averaged over 10–100 runs.

by nonspecific binding, the  $P_{RM}$  promoter is intrinsically weak and requires activation by CI at  $O_{R2}$  to compete effectively with  $P_R$ . One would therefore expect nonspecific DNA binding by CI to drastically destabilize the lysogen, and to compromise the bistability of the switch. Table S1 shows how the concentration of free CI depends on the nonspecific DNA binding strength. To characterize the effects on switch function, we investigated the range of nonspecific binding strengths over which our model gave bistability for different values of the DNA looping parameter  $\Delta G_{loop}$ . Our results are shown in Fig. 3. In the absence of DNA looping (Fig. 3, top row), bistability is indeed strongly compromised as the parameter  $\Delta G_{NSB}$  is increased in magnitude (left to right). For  $|\Delta G_{NSB}| > \sim 3$  kcal/mol, the model is no longer bistable; the lysogenic state cannot be sustained, and only the anti-immune state is stable. However, when the DNA looping interaction is included in the model, bistability is maintained over a much wider range of  $\Delta G_{NSB}$  values. In Fig. 3, the width of the green (bistable) region increases dramatically as the parameter  $|\Delta G_{loop}|$  increases. Our model therefore suggests that one role of the DNA looping interaction may be to ensure that the switch continues to function even when the level of intracellular free CI is depleted by nonspecific DNA binding (7, 8) or by cell-to-cell fluctuations (15). We note that this stability to CI fluctuations is not expected to prevent lysis from occurring on UV irradiation of the wild-type phage: even for a strong looping interaction, rapid degradation of CI by RecA will eventually lead to so little CI being present that the loop cannot be maintained, on which lysis will occur. To check this, we simulated a version of the model in which we fixed the total CI concentration, for a typical parameter set ( $\Delta G_{loop} = -3.7$  kcal/mol,  $\Delta G_{NSB} = -2.8$  kcal/mol). When we artificially lower the CI concentration to  $\approx 10\%$  of the lysogenic steady-state level (30 CI molecules per cell), the system flips to the anti-immune state. This result is in good agreement with the observation of Bailone et al. (42) that prophage induction occurs at a CI concentration  $\approx 10\%$  of that in the lysogen.

### DNA Looping Causes Robustness to Operator Mutations

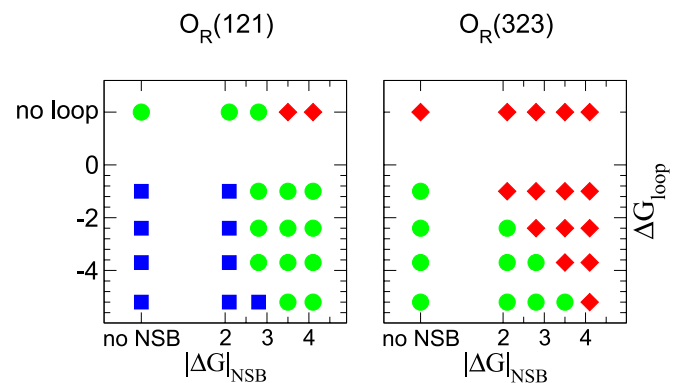
In an important series of experiments, Little et al. (3) showed that the basic functions of the phage  $\lambda$  regulatory network are robust to changes in network architecture. Mutants  $O_R(121)$  and  $O_R(323)$ , in which the order of the  $O_{R1}$ ,  $O_{R2}$ , and  $O_{R3}$  binding sites was altered compared with the wild-type  $O_R(321)$ , formed stable lysogens (although less stable than the wild type) that could be induced to enter the lytic pathway on UV irradiation (3). To our knowledge, this robustness has not been reproduced in computer models (4).

We tested whether our model was able to produce stable lysogens for the  $O_R(121)$  and  $O_R(323)$  mutants, which were created in our simulations by changing the operator binding site affinities. We neglect possible changes in the properties of  $P_{RM}$  (43), whose DNA sequence is also affected by the Little et al. (3) substitutions. The

range of parameters for which our model mutants are bistable is shown in Fig. 4. We find that the DNA looping interaction plays a key role in ensuring robustness. In the absence of DNA looping (Fig. 4, top row), our model produces a stable lysogen for  $O_R(121)$  only if the nonspecific binding strength  $\Delta G_{NSB}$  is below a critical value, and cannot sustain a stable lysogen for  $O_R(323)$  at all. However, when DNA looping is included in the model (Fig. 4, lower rows), the  $O_R(323)$  mutant can achieve a stable lysogenic state, and the  $O_R(121)$  lysogen becomes able to tolerate stronger nonspecific DNA binding interactions. In fact, if the looping strength is too strong, our model predicts that the  $O_R(121)$  mutant may lose the ability to sustain a stable anti-immune state. The steady-state concentrations of CI in the lysogen predicted for these mutants are in reasonable agreement with the observations of Little et al. (3). For a typical parameter set ( $\Delta G_{loop} = -3.7$  kcal/mol,  $\Delta G_{NSB} = -2.8$  kcal/mol), we obtained steady-state lysogenic CI concentrations 38% and 81% of the wild-type values for  $O_R(121)$  and  $O_R(323)$ , respectively, compared with 25–30% and 60–75% measured by Little et al. (3). Calculation of the spontaneous switching rates for the  $O_R(121)$  and  $O_R(323)$  mutants, using FFS, also shows results qualitatively in agreement with those of Little et al. (3) (see Table S2). For all combinations of  $\Delta G_{loop}$  and  $\Delta G_{NSB}$ , the  $O_R(323)$  lysogen is less stable than  $O_R(121)$ , which in turn is less stable than the wild type, although quantitatively the magnitude of this destabilization is greater than the factors of 10 observed by Little et al. (3). Our model allows us to make the tentative prediction that (in a *recA*–host) the order of stability of the anti-immune states will be  $O_R(323) > \text{wild-type} > O_R(121)$ . These stabilities have not yet been measured (J. Little, personal communication).

### Alternatives to Looping

Our results indicate that the DNA looping interaction allows the phage  $\lambda$  switch to maintain an extremely stable lysogenic state, which is robust to operator mutations, while retaining its essen-



**Fig. 4.** Range of bistability of the model switch as a function of the nonspecific DNA binding and DNA looping strengths, for the Little mutants. See legend to Fig. 3 for symbol definitions.



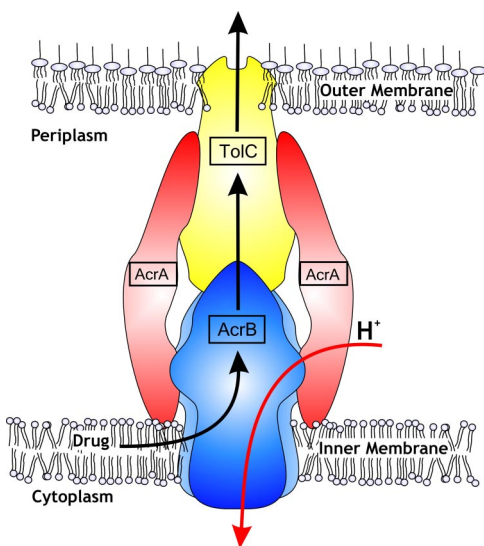
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## Corrections

### COMMENTARY

Correction for “Trinity revealed: Stoichiometric complex assembly of a bacterial multidrug efflux pump,” by Klaas M. Pos, which appeared in issue 17, April 28, 2009, of *Proc Natl Acad Sci USA* (106:6893–6894; first published April 22, 2009; 10.1073/pnas.0902837106).

The author notes that due to a printer’s error, in the legend for Fig. 1 on page 6893, the affiliation for Andrea Eberle should have appeared as “ETH Zürich, Switzerland.” In addition, in the fourth line of the legend, “inner membrane RND component AcrA” should instead read “inner membrane RND component AcrB.” The figure and corrected legend appear below. On page 6894, the final sentence of the commentary, “The AcrAB–TolC trinity would then represent machinery” should instead read “The AcrAB–TolC trinity would then represent a true peristaltic machinery.”



**Fig. 1.** Schematic drawing of tripartite RND multidrug efflux system AcrAB–TolC of the Gram-negative bacterium *Escherichia coli* (courtesy of Andrea Eberle, ETH Zürich, Switzerland). Suggestions on the stoichiometry of the adaptor AcrA to inner membrane RND component AcrB (or to outer membrane channel TolC) vary between 1 and 4.

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### CHEMISTRY, BIOPHYSICS AND COMPUTATIONAL BIOLOGY

Correction for “DNA looping provides stability and robustness to the bacteriophage  $\lambda$  switch,” by Marco J. Morelli, Pieter Rein ten Wolde, and Rosalind J. Allen, which appeared in issue 20, May 19, 2009, of *Proc Natl Acad Sci USA* (106:8101–8106; first published May 1, 2009; 10.1073/pnas.0810399106).

The authors note that due to a printer’s error, reference citations 22–50 appeared incorrectly in the text, starting on page 8102. The online version has been corrected.

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### ANTHROPOLOGY

Correction for “Economics, cultural transmission, and the dynamics of the sex ratio at birth in China,” by Mikhail Lipatov, Shuzhuo Li, and Marcus W. Feldman, which appeared in issue 49, December 9, 2008, of *Proc Natl Acad Sci USA* (105:19171–19176; first published December 1, 2008; 10.1073/pnas.0806747105).

The authors note that on page 19172, right column, fifth full paragraph, the last sentence is incorrect in part. “She then transfers this modified  $\sigma_s$  to her own daughter—the grandmother of  $A$ ,” should read “She then transfers this modified  $\sigma_s$  to her own daughter—the **granddaughter** of  $A$ .” On page 19173, in the legend for Fig. 2, the last sentence is incorrect in part. “In this mode, direction and rate of change in  $r$  is determined by the effect of parental and societal intuitive preferences ( $\rho$ ) on the transmission of cultural belief ( $\sigma_s$ ), i.e., parameters  $C_{01}$ ,  $C_{10}$ ,  $V_{01}$  and  $V_{10}$ ,” should instead read “In this mode, direction and rate of change in  $r$  is determined by the effect of parental and societal intuitive preferences ( $\rho$ ) on the transmission of cultural belief ( $\sigma_s$ ), i.e., parameters  $C_{01}$ ,  $C_{10}$ ,  $V_{01}$  and  $V_{10}$  **in Table 2.**” On page 19174, in Table 2, the last sentence of the legend, “Note that parameter subscripts refer to the direction of change in  $\sigma_s$ , not the state of parental  $\rho$ ,” should read “Note that parameter subscripts refer to the direction of change in  $\sigma_s$  **from the first subscript to the second**, not the state of parental  $\rho$ .” On page 19175, right column, first full paragraph, seventh line, “Its greatest effect ...” should read “**Most likely**, its greatest effect ...”. These errors do not affect the conclusions of the article.

[www.pnas.org/cgi/doi/10.1073/pnas.0904442106](http://www.pnas.org/cgi/doi/10.1073/pnas.0904442106)

### CELL BIOLOGY

Correction for “Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity,” by L. Vermeulen, M. Todaro, F. de Sousa Mello, M. R. Sprick, K. Kemper, M. Perez Alea, D. J. Richel, G. Stassi, and J. P. Medema, which appeared in issue 36, September 9, 2008, of *Proc Natl Acad Sci USA* (105:13427–13432; first published September 2, 2008; 10.1073/pnas.0805706105).

The authors wish to note the following: “We inadvertently overlooked an earlier report by S. C. Kirkland (28), relevant to our work.” The related reference citation appears below.

28. Kirkland SC (1988) Clonal origin of columnar, mucous, and endocrine cell lineages in human colorectal epithelium. *Cancer* 61:1359–1363.

[www.pnas.org/cgi/doi/10.1073/pnas.0904353106](http://www.pnas.org/cgi/doi/10.1073/pnas.0904353106)