Report

Current Biology

Bacterial Autoimmunity Due to a Restriction-Modification System

Highlights

- Restriction-modification (RM) systems occasionally cleave self-DNA (self-restriction)
- Individual bacteria can recover from self-restriction and maintain viability
- More efficient RM systems are more likely to cause self-restriction
- Stochastic events contribute to the overall rate of self-restriction

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In Brief

Restriction-modification (RM) systems protect nearly all prokaryotes from parasitic DNA. Pleška et al. show that a subpopulation of bacteria carrying an RM system suffers from autoimmunity—a stochastic process, which temporarily disrupts the host's genome integrity.

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Bacterial Autoimmunity Due to a Restriction-Modification System

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SUMMARY

Restriction-modification (RM) systems represent a minimal and ubiquitous biological system of self/non-self discrimination in prokaryotes [1], which protects hosts from exogenous DNA [2]. The mechanism is based on the balance between methyltransferase (M) and cognate restriction endonuclease (R). M tags endogenous DNA as self by methylating short specific DNA sequences called restriction sites, whereas R recognizes unmethylated restriction sites as non-self and introduces a double-stranded DNA break [3]. Restriction sites are significantly underrepresented in prokaryotic genomes [4–7], suggesting that the discrimination mechanism is imperfect and occasionally leads to autoimmunity due to self-DNA cleavage (self-restriction) [8]. Furthermore, RM systems can promote DNA recombination [9] and contribute to genetic variation in microbial populations, thus facilitating adaptive evolution [10]. However, cleavage of self-DNA by RM systems as elements shaping prokaryotic genomes has not been directly detected, and its cause, frequency, and outcome are unknown. We quantify self-restriction caused by two RM systems of *Escherichia coli* and find that, in agreement with levels of restriction site avoidance, EcoRI, but not EcoRV, cleaves self-DNA at a measurable rate. Self-restriction is a stochastic process, which temporarily induces the SOS response, and is followed by DNA repair, maintaining cell viability. We find that RM systems with higher restriction efficiency against bacteriophage infections exhibit a higher rate of self-restriction, and that this rate can be further increased by stochastic imbalance between R and M. Our results identify molecular noise in RM systems as a factor shaping prokaryotic genomes.

RESULTS AND DISCUSSION

EcoRI, but Not EcoRV, Induces DNA Damage in Host Bacteria

We hypothesized that natively occurring restriction-modification (RM) systems cause occasional self-restriction and that this is detrimental to their host bacteria. To test this hypothesis, we compared population doubling times of *Escherichia coli* MG1655 (wild-type) with plasmids carrying EcoRI or EcoRV RM systems (R+M+) expressed from their native promoters, respective control plasmids deficient in R activity (R−M+), and the plasmid backbone control (R−M−) (Figure S1A). Population doubling times of cells carrying EcoRI and EcoRV (R+M+) plasmids did not significantly differ from the controls (Table 1), indicating that self-restriction is either rare and/or its effect is small due to the ability of wild-type cells to repair DNA damage [11, 12]. We observed no measurable fitness effect in direct competitions between (R+M+) and (R−M+) strains of EcoRI and EcoRV in rich medium (M9, 0.4% glucose, 0.2% casamino acids) (Figure S1B) but observed decreased fitness due to EcoRI (R+M+) in minimal medium (M9, 0.4% glucose) (Figure S1C). Earlier studies have shown that induced chronic double-stranded DNA breaks occurring once per replication cycle have only a small effect (0.6%) on the proliferation rate of wild-type *E. coli* [13], and that the capacity to repair DNA damage is limited by resource availability [14]. To test whether DNA damage occurs at elevated levels in populations carrying EcoRI, we measured the population doubling time of the *recA* knockout (Δ*recA*) strain carrying the RM plasmids. RecA is an essential component of DNA repair and, unlike wild-type cells, *recA* mutants are sensitive to self-restriction provoked by artificially induced imbalance between R and M expression (Figure S2A). Deleting *recA* increased the population doubling time of all strains by approximately 6 min (15% of the wild-type doubling time) (Table 1), reflecting the inability of Δ*recA* cells to repair spontaneous DNA damage [15]. Presence of the plasmid expressing EcoRI (R+M+) increased the doubling time of Δ*recA* cells significantly by an additional 3 min as compared to the EcoRI (R−M+) control. In contrast, the EcoRV (R+M+) plasmid had no statistically significant effect on growth of the Δ*recA* strain.

Table 1. Population Doubling Times of Strains Carrying Wild-Type EcoRI and EcoRV RM Systems

Plasmid	PDT (min)[a]	SD (min)[a]	p Value[a,b]
Host: Wild-Type			
Control (R−M−)	40.09	1.49	–
EcoRI (R+M+)	40.78	0.68	0.429
EcoRI (R−M+)	40.68	0.84	0.492
EcoRV (R+M+)	40.79	0.57	0.422
EcoRV (R−M+)	40.19	1.19	0.908
Host: Δ*recA*			
Control (R−M−)	46.06	0.59	–
EcoRI (R+M+)	48.98	0.75	0.003
EcoRI (R−M+)	46.20	0.99	0.854
EcoRV (R+M+)	46.84	1.14	0.327
EcoRV (R−M+)	45.67	1.08	0.626

PDT, population doubling time.

[a]Calculated from three independent experiments, each with six biological replicates.

[b]The p values were calculated by linear regression, with the population doubling time as a continuous dependent variable and strain identity as a categorical independent variable, comparing individual strains to the control (R−M−).

The results suggested that EcoRI increased the amount of DNA damage in the population and that RecA alleviated most of the negative effect on growth. The mechanism of restriction alleviation, which prevents self-restriction by type I RM systems, is unlikely to affect our estimates, because both EcoRI and EcoRV are type II RM systems and therefore are insensitive to restriction alleviation [16].

EcoRI, but Not EcoRV, Induces the SOS Response in a Subpopulation of Host Bacteria

We next investigated whether the increased amount of DNA damage due to EcoRI can be explained by higher frequency of self-restriction as compared to EcoRV. We quantified the fraction of cells suffering from DNA damage in populations carrying the two RM systems using flow cytometry and a reporter strain with a fast-maturing yellow fluorescent protein (YFP) [17] fused to the promoter of *sulA* (P_{sulA}-*yfp*). SulA is strongly upregulated as a part of the SOS response, a global stress response to DNA damage in *E. coli* [18]. Similar P_{sulA}-based reporters have been previously used to quantify the extent of DNA damage in bacteria [13, 15, 19]. Self-restriction provoked by artificially induced imbalance between R and M strongly increased fluorescence of individual cells as a result of SOS response induction (Figure S2B). When EcoRI (R+M+) was expressed from its native promoters, the population contained more highly fluorescent cells as compared to the controls (Figure 1A), showing that self-restriction occurred in a subpopulation of cells and induced the SOS response in this subpopulation. No such effect was observed for cells carrying EcoRV. We quantified the fraction of cells with induced SOS response (SOS-ON) for each strain by first quantifying the fraction of cells with fluorescence above a threshold. The threshold was chosen based on the location at which the cumulative tail probability distribution of the wild-type population changes slope, corresponding to the point at which SOS-OFF and SOS-ON subpopulations begin to overlap (Figure 1B). Because RecA is necessary for induction of the SOS response [18], Δ*recA* populations did not show such a change in slope. The threshold value was consistent across all samples (Figure 1C). The wild-type and Δ*recA* control (R−M−) populations contained 0.92% and 0.35% cells with fluorescence above the threshold, respectively (Figure 1D; Table S1). Subtracting the background fraction of cells above the threshold in the Δ*recA* control from the fraction of cells above the threshold in the wild-type gave an estimate of 0.57% cells being genuinely SOS-ON as a result of spontaneous DNA damage. This is in rough agreement with the previously estimated fraction of 0.9% cells being SOS-ON due to spontaneous DNA damage under slightly different growth conditions [15]. Using the same method and threshold value, the EcoRI (R+M+) populations contained 0.91% genuine SOS-ON cells, which corresponds to a significant 1.6-fold increase as compared to the EcoRI (R−M+) population (Figure 1D; Table S1). The effect of the EcoRV (R+M+) plasmid on the number of SOS-ON cells was not significant, which is consistent with our observation of EcoRI, but not EcoRV, inducing DNA damage in host bacteria. It is possible that the estimated fraction of cells suffering from self-restriction by EcoRI is an underestimate, because EcoRI, unlike EcoRV, generates cohesive ends that can be directly ligated by DNA ligase before induction of the SOS response takes place [20].

SOS Response Is Stochastically and Dynamically Turned On and Off in Cells Suffering from Self-Restriction

To monitor the fate of cells undergoing self-restriction, we observed single cells carrying EcoRI in real time using fluorescence long-term time-lapse microscopy. We measured the levels of P_{sulA}-*yfp* expression in single cells growing in steady state inside a microfluidic device (Figure 2A; Movie S1). Because RecA is necessary for SOS induction [18], we first determined the threshold of fluorescence above which cells are evaluated as SOS-ON using a Δ*recA* control. Cellular fluorescence of Δ*recA* cells fluctuated due to noise, but no sharp increase was observed in contrast to wild-type cells (Figure S3A). We set the threshold accordingly and calculated the frequency at which fluorescence intensity of wild-type cells carrying the EcoRI RM system crossed the threshold (Figure 2B; Table S2). At the threshold value of 75 (a.u.), the wild-type restriction-deficient strains [control (R−M−) and EcoRI (R−M+)] displayed nearly identical frequency of SOS induction: $(2.7 \pm 0.4) \times 10^{-3}$ min^{-1} and $(2.8 \pm 0.4) \times 10^{-3}$ min^{-1}, respectively, as a result of spontaneous DNA damage. The EcoRI (R+M+) strain induced SOS response at the rate of $(4.7 \pm 0.4) \times 10^{-3}$ min^{-1}, which corresponds to a 1.7-fold increase (Figure 2C). In total, we observed 0.53% and 0.94% cells being SOS-ON in the control (R−M−) and EcoRI (R+M+) populations, respectively (Table S2), which is in agreement with the flow-cytometry experiments. Using a threshold value of 100 (a.u.) did not affect the result qualitatively [1.4-fold increase in SOS induction frequency of EcoRI (R+M+) cells]. Interestingly, the SOS-ON cells in the EcoRI (R+M+) as well as EcoRI (R−M+) and control (R−M−) populations returned rapidly to

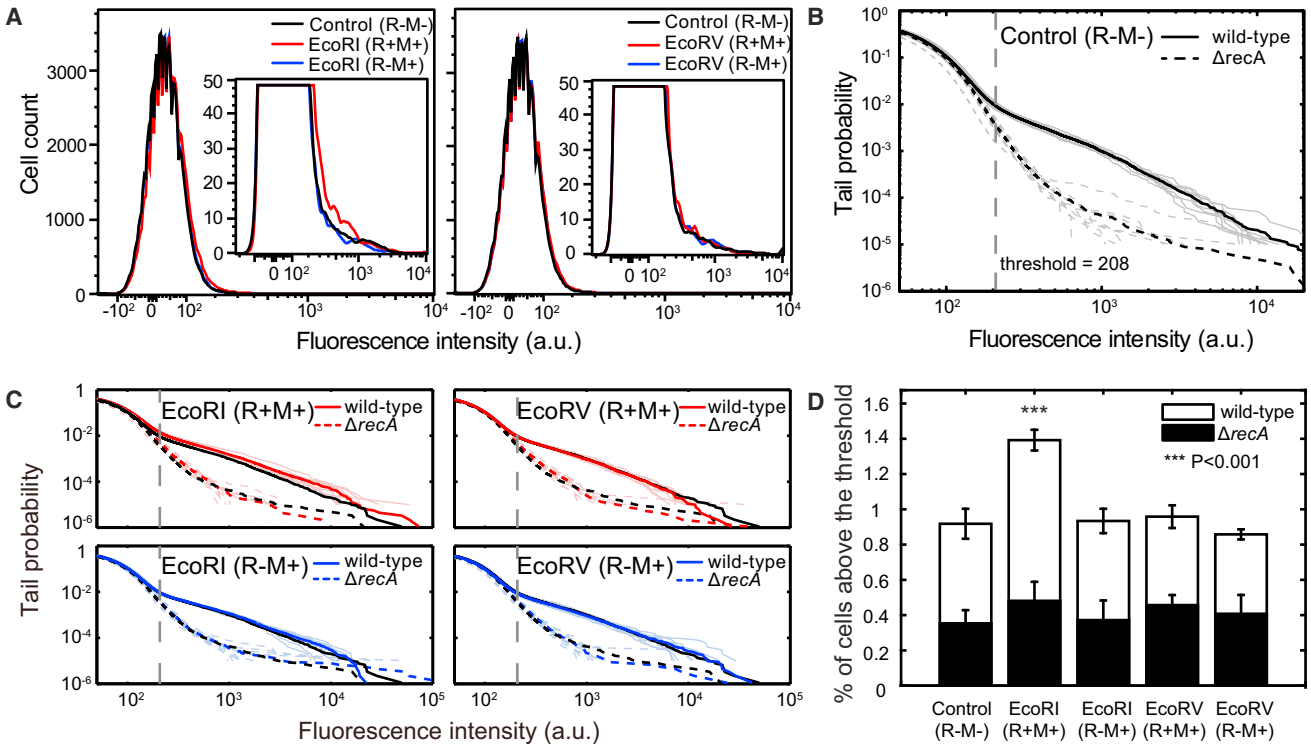

Figure 1. EcoRI, but Not EcoRV, Induces the SOS Response in a Subpopulation of Cells Due to Self-Restriction

(A) Representative flow-cytometry histograms of cell fluorescence, corresponding to the YFP expressed from the P_{sulA} promoter in the presence of EcoRI and EcoRV RM systems expressed from their native promoters. EcoRI increases the number of highly fluorescent cells as a consequence of self-restriction. EcoRV has no effect. The insets show the same data on a rescaled y axis to emphasize the tail behavior. 100,000 cells were measured in each sample. The x axes were biexponentially transformed.

(B) The threshold value used to quantify the fractions of SOS-ON cells was picked based on the log-log plot of cumulative tail probability versus fluorescence. 100,000 cells were measured in each sample. Nine biological replicates from three independent experiments are shown as gray lines. Thick black lines represent pooled data. The vertical gray dashed line represents the threshold value = 208 (a.u.).

(C) The threshold value = 208 (a.u.) (vertical gray dashed lines) was consistent for all strains. The data are plotted as in (B), with pale lines representing nine biological replicates obtained in three independent experiments (100,000 cells measured in each sample). Thick colored lines represent pooled data. The thick black lines represent pooled control (R−M−) populations.

(D) Fractions of cells with fluorescence above the threshold in populations carrying RM systems expressed from their native promoters. The error bars represent the SD between three experimental averages (three biological replicates each). Asterisks indicate the level of significance. The p values were calculated by linear regression, with the number of cells above the threshold as a continuous dependent variable and strain identity as a categorical independent variable, comparing individual strains to the control (R−M−). The number of genuine SOS-ON cells can be obtained by subtracting the background fraction of cells above the threshold in the Δ*recA* control from the fraction of cells above the threshold in the wild-type.

See also Figure S2 and Table S1.

the SOS-OFF state and continued to grow and divide normally (Figure 2A; Movie S1). P_{sulA}-*yfp* induction was not associated with filamentation or cell death, and the level of SOS response induction did not correlate with the single-cell elongation rate (Figure S3B) or generation time (Figure S3C). These results indicated that under our experimental conditions, wild-type cells growing in steady state repair the DNA damage caused by both self-restriction and spontaneous DNA damage with high efficiency and thus remain in the growing population. In our experiments, self-restriction occurs during stable maintenance of the RM system and induces the SOS response only transiently, without affecting the viability of individual cells. This stands in sharp contrast to the previously described process of postsegregational killing [21], which occurs when intracellular levels of R and M are irreversibly disturbed by dilution following gene loss [22] and ultimately leads to cell death [23].

The Rate of Self-Restriction Is Higher for More Efficient RM Systems and Can Be Increased by Stochastic Imbalance between R and M

Our experiments show that the probability of self-restriction is higher for EcoRI than EcoRV. Interestingly, this does not correspond to the number of restriction sites that are potential targets for self-restriction in the genome of *E. coli* MG1655 (599 GAATTC for EcoRI and 1,888 GATATC for EcoRV) but agrees with the estimated levels of restriction site avoidance (Figure S4A). The EcoRI restriction site frequency is reduced by 50% from its expected value, whereas the EcoRV site is slightly enriched compared to expectation. This difference in EcoRI and EcoRV restriction site frequencies was previously noticed [5] although not explained. We hypothesized that the difference in self-restriction rate between the two RM systems results from an intrinsic difference in restriction efficiency per single restriction

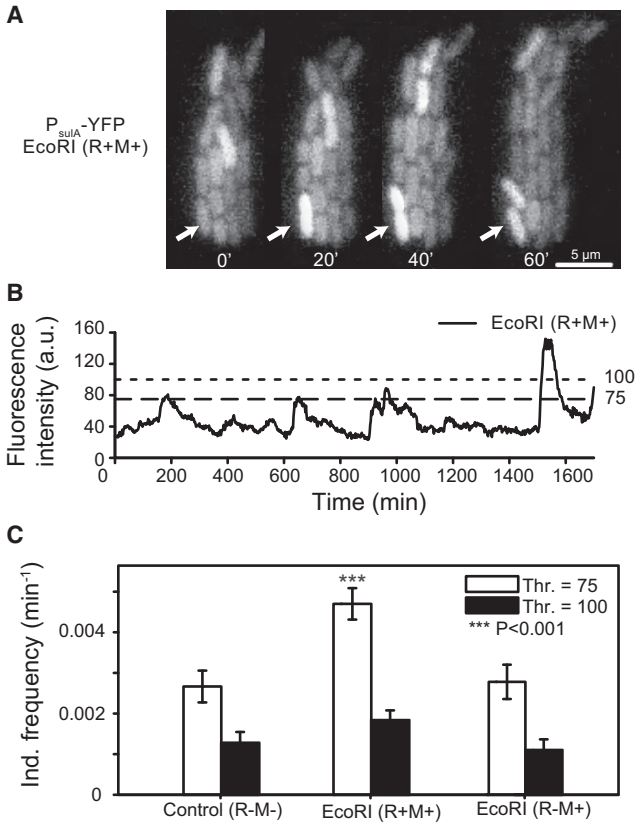

Figure 2. Real-Time Dynamics of Self-Restriction by EcoRI in Single Cells

(A) Representative time-lapse images showing spontaneous temporary induction of YFP expressed from the P_{sulA} promoter in wild-type cells carrying the EcoRI (R+M+) RM system. Cells growing inside a microfluidic device show spontaneous induction of the SOS response, followed by dilution of YFP due to cell division. Arrows point to the cell that underwent SOS induction and subsequent cell division.

(B) Representative single-cell lineage showing dynamics of YFP expression from the P_{sulA} promoter of the EcoRI (R+M+) strain. The fluorescence intensity at each time point is evaluated as the mean pixel brightness within a region corresponding to a single cell subtracted by the background brightness. The horizontal dashed lines indicate the threshold levels used to calculate SOS induction frequencies.

(C) SOS induction frequencies calculated as the total counts of fluorescence intensity crossing the respective threshold values divided by the total time length of all the branches in the lineage trees, on which we found 463 control (R−M−), 866 EcoRI (R+M+), and 465 EcoRI (R−M+) cells, including those flown away from the growth channels before division. The error bars indicate the binomial errors in calculating the induction frequency.

See also Figure S3, Table S2, and Movie S1.

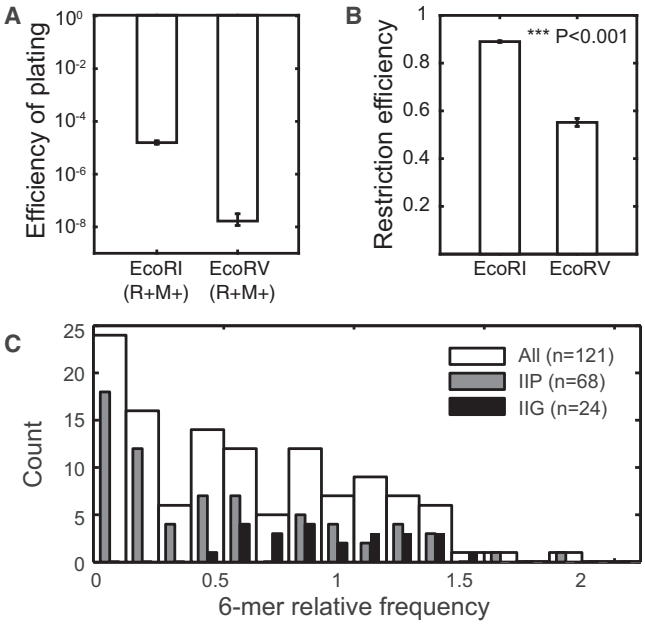

Figure 3. Identifying Determinants of Self-Restriction

(A) Efficiency of plating of λ *vir* on the lawns of cells with respective RM systems, corresponding to the probability that the phage will not be restricted by the RM system. λ *vir* contains 5 EcoRI and 22 EcoRV restriction sites. The error bars represent the SD calculated from four independent experiments. See also Figure S4B.

(B) Efficiencies of restriction per restriction site. The values were calculated from the data shown in (A). The error bars represent the SD calculated from four independent experiments. The p value was calculated with the t test ($t = 39.6$ on 6 degrees of freedom, $p < 10^{-4}$).

(C) For all predicted six-cutter RM systems in the stringent set, the distribution of relative frequencies of putative restriction sites in the assigned host genomes is shown as white bars. Relative frequencies of 6-bp restriction sites of type IIG (where R and M are fused into a single bifunctional unit) and the canonical type IIP (where R and M are structurally and functionally independent) RM systems are shown as black and gray bars, respectively. The 29 remaining RM systems are of type IIS, type IIF, or solitary endonucleases, and are not shown as separate bars. See also Figure S4A and Data S1.

site (the probability that a restriction site is cleaved before methylation). We tested this hypothesis by measuring the efficiency of EcoRI and EcoRV in preventing infection by unmethylated bacteriophage λ *vir*. The efficiency of plating (*eop*), reflecting the probability of λ *vir* escaping restriction, was 1.6×10^{-5} and 2.7×10^{-8} for EcoRI and EcoRV, respectively (Figure 3A). Assuming that a phage with n restriction sites (5 for EcoRI and 22 for EcoRV in λ *vir* [24]) escapes restriction when all its restriction sites are methylated before cleavage occurs [25], the restriction efficiency per restriction site is given by $1 - \sqrt[n]{eop}$. In agreement with our hypothesis, the restriction efficiency is significantly higher for EcoRI than EcoRV (Figure 3B). Neither EcoRI nor EcoRV restricted fully methylated λ *vir* (Figure S4B), indicating that under our experimental conditions, EcoRI and EcoRV do not cleave DNA at methylated or noncognate restriction sites [26]. Their different restriction efficiencies thus most likely reflect a difference in R and M gene expression levels and enzymatic activities. These results suggest that RM systems with higher per-site probability of cleavage are more likely to cause self-restriction and lead to stronger restriction site avoidance. Although a variety of gene regulatory mechanisms are known to maintain well-balanced levels of R and M expression [27–31], stochastic events occurring at the level of the single cell, such as stochastic gene expression [32] or protein partitioning at cell division [33], might occasionally disrupt this balance and contribute to the overall rate of self-restriction. In support of this hypothesis, we found that restriction sites of type IIP RM systems, in which R and M are structurally and functionally independent enzymes, were on average more avoided

and exhibited a wider range of genomic frequencies than restriction sites of type IIG RM systems, in which R and M are fused into a single bifunctional polypeptide [34] (Figure 3B). The direct linkage of R and M will minimize the probability of stochastic R and M imbalance due to fluctuations of individual components, which is expected to reduce self-restriction rates. In contrast, self-restriction in type IIP systems (which include EcoRI and EcoRV) can result from differences in expression levels and enzymatic activities of R and M as well as stochastic imbalance between their concentrations. This additional source of variance in type IIP systems is consistent with the significantly wider range of their restriction site frequencies and higher avoidance on average.

Our finding that a more efficient RM system exhibits a higher self-restriction rate is indicative of an evolutionary trade-off between enhanced protection against exogenous DNA and increased autoimmunity. Evolution of restriction site avoidance in a genome mitigates the long-term cost of an RM system, which was previously estimated for six-cutter enzymes to be 10^{-5} to 10^{-4} per generation at mutation-selection balance [8]. Although we did not observe a measurable fitness cost of self-restriction under standard conditions, we did observe a noticeable fitness cost when resources were limited. The long-term cost of RM systems in natural populations will thus depend both on molecular properties of individual RM systems and on environmental determinants. These results are in accord with studies showing that other phage resistance mechanisms such as clustered regularly interspaced short palindromic repeats (CRISPR) loci, coupled to CRISPR-associated genes (CRISPR-Cas) [35, 36], abortive infection [37, 38], or envelope resistance [39, 40] come with a cost for the host cell, and that the cost of immunity can be accentuated in environments with limited resource availability in bacteria [41], as well as in higher organisms [42].

The ability to discriminate self from non-self is a crucial property of all immune systems [43], and failure to do so leads to pathogen tolerance or autoimmunity [44]. In this work, we show that similar to more complex immune systems, autoimmunity due to RM systems affects a small number of individuals in a population. RM systems are extremely abundant in prokaryotes [1, 45], and most likely play an important role in their ecology and evolution. Understanding the costs and benefits associated with RM systems is crucial to fully evaluate this role [46]. RM systems can protect their hosts from parasites [3] but also act parasitically [21]. Although they act as a barrier to horizontal gene transfer [2, 47], they themselves are often mobile [48] and can even promote DNA recombination [49]. Here we describe a new type of interaction between RM systems and their hosts—a primitive form of bacterial autoimmunity. As a downside of an immunity mechanism based on a balance between individual components, bacterial self-restriction exemplifies a link between stochastic events occurring at the level of single individuals and the evolution of bacterial genomes.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, two tables, one movie, and one dataset and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.12.041.

AUTHOR CONTRIBUTIONS

T.B., C.C.G., E.K., M.P., L.Q., and Y.W. designed the research. T.B. and M.P. constructed strains and plasmids. R.O. and M.P. performed experiments. L.Q. performed the bioinformatics analysis. M.P., L.Q., and Y.W. analyzed the data. T.B., C.C.G., E.K., M.P., L.Q., and Y.W. wrote the paper.

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REFERENCES

1. Oliveira, P.H., Touchon, M., and Rocha, E.P.C. (2014). The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. Nucleic Acids Res. *42*, 10618–10631.
2. Murray, N.E. (2002). 2001 Fred Griffith Review Lecture. Immigration control of DNA in bacteria: self versus non-self. Microbiology *148*, 3–20.
3. Arber, W., and Dussoix, D. (1962). Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage lambda. J. Mol. Biol. *5*, 18–36.
4. Karlin, S., Burge, C., and Campbell, A.M. (1992). Statistical analyses of counts and distributions of restriction sites in DNA sequences. Nucleic Acids Res. *20*, 1363–1370.
5. Gelfand, M.S., and Koonin, E.V. (1997). Avoidance of palindromic words in bacterial and archaeal genomes: a close connection with restriction enzymes. Nucleic Acids Res. *25*, 2430–2439.
6. Rocha, E.P.C., Danchin, A., and Viari, A. (2001). Evolutionary role of restriction/modification systems as revealed by comparative genome analysis. Genome Res. *11*, 946–958.
7. Elhai, J. (2001). Determination of bias in the relative abundance of oligonucleotides in DNA sequences. J. Comput. Biol. *8*, 151–175.
8. Qian, L., and Kussell, E. (2012). Evolutionary dynamics of restriction site avoidance. Phys. Rev. Lett. *108*, 158105.
9. Chang, S., and Cohen, S.N. (1977). In vivo site-specific genetic recombination promoted by the EcoRI restriction endonuclease. Proc. Natl. Acad. Sci. USA *74*, 4811–4815.
10. Asakura, Y., Kojima, H., and Kobayashi, I. (2011). Evolutionary genome engineering using a restriction-modification system. Nucleic Acids Res. *39*, 9034–9046.
11. Heitman, J., Ivanenko, T., and Kiss, A. (1999). DNA nicks inflicted by restriction endonucleases are repaired by a RecA- and RecB-dependent pathway in *Escherichia coli*. Mol. Microbiol. *33*, 1141–1151.
12. Cromie, G.A., and Leach, D.R.F. (2001). Recombinational repair of chromosomal DNA double-strand breaks generated by a restriction endonuclease. Mol. Microbiol. *41*, 873–883.
13. Darmon, E., Eykelenboom, J.K., Lopez-Vernaza, M.A., White, M.A., and Leach, D.R.F. (2014). Repair on the go: *E. coli* maintains a high proliferation rate while repairing a chronic DNA double-strand break. PLoS ONE *9*, e110784.

14. Sargentini, N.J., Diver, W.P., and Smith, K.C. (1983). The effect of growth conditions on inducible, recA-dependent resistance to X rays in *Escherichia coli*. Radiat. Res. *93*, 364–380.
15. Pennington, J.M., and Rosenberg, S.M. (2007). Spontaneous DNA breakage in single living *Escherichia coli* cells. Nat. Genet. *39*, 797–802.
16. Makovets, S., Powell, L.M., Titheradge, A.J.B., Blakely, G.W., and Murray, N.E. (2004). Is modification sufficient to protect a bacterial chromosome from a resident restriction endonuclease? Mol. Microbiol. *51*, 135–147.
17. Nagai, T., Ibata, K., Park, E.S., Kubota, M., Mikoshiba, K., and Miyawaki, A. (2002). A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. Nat. Biotechnol. *20*, 87–90.
18. Friedberg, E.C., Walker, G.C., Siede, W., and Wood, R.D. (2005). DNA Repair and Mutagenesis (American Society for Microbiology Press).
19. Handa, N., Ichige, A., Kusano, K., and Kobayashi, I. (2000). Cellular responses to postsegregational killing by restriction-modification genes. J. Bacteriol. *182*, 2218–2229.
20. Heitman, J., Zinder, N.D., and Model, P. (1989). Repair of the *Escherichia coli* chromosome after in vivo scission by the EcoRI endonuclease. Proc. Natl. Acad. Sci. USA *86*, 2281–2285.
21. Naito, T., Kusano, K., and Kobayashi, I. (1995). Selfish behavior of restriction-modification systems. Science *267*, 897–899.
22. Ichige, A., and Kobayashi, I. (2005). Stability of EcoRI restriction-modification enzymes in vivo differentiates the EcoRI restriction-modification system from other postsegregational cell killing systems. J. Bacteriol. *187*, 6612–6621.
23. Asakura, Y., and Kobayashi, I. (2009). From damaged genome to cell surface: transcriptome changes during bacterial cell death triggered by loss of a restriction-modification gene complex. Nucleic Acids Res. *37*, 3021–3031.
24. Roberts, R.J., Vincze, T., Posfai, J., and Macelis, D. (2015). REBASE—a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. *43*, D298–D299.
25. Enikeeva, F.N., Severinov, K.V., and Gelfand, M.S. (2010). Restriction-modification systems and bacteriophage invasion: who wins? J. Theor. Biol. *266*, 550–559.
26. Vasu, K., Nagamalleswari, E., and Nagaraja, V. (2012). Promiscuous restriction is a cellular defense strategy that confers fitness advantage to bacteria. Proc. Natl. Acad. Sci. USA *109*, E1287–E1293.
27. Semenova, E., Minakhin, L., Bogdanova, E., Nagornykh, M., Vasilov, A., Heyduk, T., Solonin, A., Zakharova, M., and Severinov, K. (2005). Transcription regulation of the EcoRV restriction-modification system. Nucleic Acids Res. *33*, 6942–6951.
28. Nagornykh, M.O., Bogdanova, E.S., Protsenko, A.S., Zakharova, M.V., Solonin, A.S., and Severinov, K.V. (2008). [Regulation of gene expression in type II restriction-modification system]. Genetika *44*, 606–615.
29. Mruk, I., and Blumenthal, R.M. (2008). Real-time kinetics of restriction-modification gene expression after entry into a new host cell. Nucleic Acids Res. *36*, 2581–2593.
30. Mruk, I., Liu, Y., Ge, L., and Kobayashi, I. (2011). Antisense RNA associated with biological regulation of a restriction-modification system. Nucleic Acids Res. *39*, 5622–5632.
31. Mruk, I., and Kobayashi, I. (2014). To be or not to be: regulation of restriction-modification systems and other toxin-antitoxin systems. Nucleic Acids Res. *42*, 70–86.
32. Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic gene expression in a single cell. Science *297*, 1183–1186.
33. Huh, D., and Paulsson, J. (2011). Non-genetic heterogeneity from stochastic partitioning at cell division. Nat. Genet. *43*, 95–100.
34. Roberts, R.J., Belfort, M., Bestor, T., Bhagwat, A.S., Bickle, T.A., Bitinaite, J., Blumenthal, R.M., Degtyarev, S.Kh., Dryden, D.T., Dybvig, K., et al. (2003). A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes. Nucleic Acids Res. *31*, 1805–1812.
35. Westra, E.R., van Houte, S., Oyesiku-Blakemore, S., Makin, B., Broniewski, J.M., Best, A., Bondy-Denomy, J., Davidson, A., Boots, M., and Buckling, A. (2015). Parasite exposure drives selective evolution of constitutive versus inducible defense. Curr. Biol. *25*, 1043–1049.
36. Vale, P.F., Lafforgue, G., Gatchitch, F., Gardan, R., Moineau, S., and Gandon, S. (2015). Costs of CRISPR-Cas-mediated resistance in *Streptococcus thermophilus*. Proc. R. Soc. B *282*, 20151270.
37. Berngruber, T.W., Lion, S., and Gandon, S. (2013). Evolution of suicide as a defence strategy against pathogens in a spatially structured environment. Ecol. Lett. *16*, 446–453.
38. Refardt, D., Bergmiller, T., and Kümmerli, R. (2013). Altruism can evolve when relatedness is low: evidence from bacteria committing suicide upon phage infection. Proc. R. Soc. B *280*, 20123035.
39. Lenski, R.E., and Levin, B.R. (1985). Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. Am. Nat. *125*, 585–602.
40. Lenski, R.E. (1988). Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. Variation in competitive fitness among mutants resistant to virus T4. Evolution *42*, 425–432.
41. Gómez, P., and Buckling, A. (2011). Bacteria-phage antagonistic coevolution in soil. Science *332*, 106–109.
42. Boots, M. (2011). The evolution of resistance to a parasite is determined by resources. Am. Nat. *178*, 214–220.
43. Marraffini, L.A., and Sontheimer, E.J. (2010). Self versus non-self discrimination during CRISPR RNA-directed immunity. Nature *463*, 568–571.
44. Goldberg, G.W., and Marraffini, L.A. (2015). Resistance and tolerance to foreign elements by prokaryotic immune systems—curating the genome. Nat. Rev. Immunol. *15*, 717–724.
45. Vasu, K., and Nagaraja, V. (2013). Diverse functions of restriction-modification systems in addition to cellular defense. Microbiol. Mol. Biol. Rev. *77*, 53–72.
46. Korona, R., and Levin, B.R. (1993). Phage-mediated selection and the evolution and maintenance of restriction-modification. Evolution *47*, 556–575.
47. Corvaglia, A.R., François, P., Hernandez, D., Perron, K., Linder, P., and Schrenzel, J. (2010). A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical *Staphylococcus aureus* strains. Proc. Natl. Acad. Sci. USA *107*, 11954–11958.
48. Furuta, Y., and Kobayashi, I. (2012). Restriction-modification systems as mobile epigenetic elements. In Bacterial Integrative Mobile Genetic Elements, A. Roberts, and P. Mullany, eds. (Landes Bioscience), pp. 1–19.
49. Arber, W. (2000). Genetic variation: molecular mechanisms and impact on microbial evolution. FEMS Microbiol. Rev. *24*, 1–7.