Gene regulation, phenotypic memory, and selection in fluctuating environments

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Abstract

Gene regulatory networks enable bacteria to sense and respond in fluctuating environments and to control gene expression levels in constant conditions. Despite substantial advances in understanding their molecular biology, the evolutionary conditions that select for and maintain gene regulatory networks have remained elusive. Here, we investigate the evolutionary costs and benefits of gene regulation under metabolic fluctuations, and identify distinct fluctuating environments that select for or against gene regulation. Using barcode sequencing to track strain frequencies over time, we compete strains with perturbed expression dynamics to assess the strength of selection on gene expression levels. We reveal that expression levels can be shaped to enhance phenotypic memory of past environmental states, reducing the impact of lag phases on long-term population growth. By independently perturbing the ability to sense the environment and the control of expression levels, we discover sign epistasis between sensing and control in a gene regulatory network, and identify its molecular underpinnings. Due to this epistatic interaction, maintenance of sensing in gene regulation enhances the ability of evolution to tune gene expression in a fluctuating environment. Our work establishes a new basis for understanding how gene regulatory networks evolve in fluctuating environments.

Introduction

Gene regulation functions to activate or repress gene expression when environments change, and to control expression levels when conditions remain constant. While these two distinct functions – sensing and control – are understood in molecular detail in different model systems [1-5], how fluctuating environments select for and maintain gene regulation during evolution has not been elucidated [6, 7]. The ability to sense and respond to fluctuations can be lost during episodes in which conditions remain constant for sufficiently long times, an outcome that has been observed in evolution experiments [8-10]. For example, *Escherichia coli* evolving in constant lactose conditions lose the ability to regulate the *lac* operon, and express the *lac* genes constitutively, a result that has been repeatedly observed both *in vitro* [8] and *in vivo* [11]. Surprisingly, however, the same occurs when bacteria evolve in a periodic environment that alternates daily between lactose and glucose: mutations in the *lac* repressor (*lacI*) sweep to fixation and evolved strains lose the ability to respond to lactose fluctuations [8, 12]. While constitutive *lac* expression can be costly [13-15], in a fluctuating environment having to turn genes on and off repeatedly can lead to lag phases [16] which are detrimental to long-term growth. However, the *lac* operon regulatory architecture is highly conserved in nature [17], indicating that natural selection acts to maintain gene regulation in this metabolic system. Understanding how cost-benefit tradeoffs play out in a fluctuating environment is therefore critical to predicting whether gene regulation will be selected for and maintained in nature [18, 19].

To achieve uninterrupted growth in a fluctuating environment, while avoiding the cost of constitutive expression, microorganisms combine gene regulation with memory mechanisms [20-24]. In alternating glucose-lactose fluctuations, *E. coli* cells 'remember' their past metabolic states to avoid lag phases each time lactose is encountered, a behavior known as phenotypic memory [24]. Phenotypic memory in the *lac* operon relies on a simple molecular mechanism: the inheritance from mother to daughter cells of stable metabolic proteins, the enzyme β-galactosidase (LacZ) and the lactose permease (LacY), which together enable growth on lactose. Phenotypic memory was shown to eliminate lag phases up to 4 generations after lactose was first encountered, or to shorten the duration of the lag phase up to 10 generations after initial exposure [24]. Modulating the expression levels of the *lac* operon, which are controlled by both the Lac repressor (LacI) and the cyclic-AMP receptor protein (Crp), is thus predicted to alter the duration of memory. While previous studies have considered how control of gene expression levels impacts bacterial fitness in constant environments [13-15, 25, 26], the molecular mechanism of phenotypic memory suggests that gene expression levels may in fact be tuned to provide memory

to future generations in a fluctuating environment. We therefore hypothesize that, depending on the period of environmental fluctuations, selection could act to tune gene expression levels and enhance phenotypic memory.

Given the intricate relationships between gene expression, phenotypic memory, and cost-benefit tradeoffs in a fluctuating environment, understanding the adaptive advantage of gene regulation requires analyzing the molecular underpinnings of growth rates and lag phases, as well as their dependence on environmental conditions and fluctuation timescales. Importantly, these must be assessed in mutational contexts that perturb distinct features of a gene regulatory network, e.g. abolishing the ability to sense and respond, altering maximal expression levels, reducing the speed of induction, etc. Here, we systematically analyze these relationships using targeted perturbations of gene regulation in the *lac* operon. Using bulk population and microfluidic growth rate measurements, we determine which fluctuating environments select for or against gene regulation. By competing strains with perturbed gene regulation and altered levels of phenotypic memory, and tracking strain frequency dynamics using barcode sequencing in a fluctuating environment, we measure the strength of selection acting on gene regulation and tuning expression levels. Our findings establish fundamental principles of how evolution shapes gene regulation and memory in bacteria, and identify the critical molecular components that underlie cost-benefit tradeoffs in fluctuating conditions. Our work bridges key gaps in understanding the selective constraints that underlie the evolution of gene regulatory networks, and has broader implications in studies of microbial communities [27], synthetic biology [4], metabolism [5], and metabolic engineering [28].

Results

Selection for phenotypic memory

To determine conditions that select for phenotypic memory, we used a library of *E. coli* strains in which we destabilized the β-galactosidase enzyme with ssrA degradation tags spanning a range of degradation rates [29]. Despite reduction of β-galactosidase expression levels by up to 30% during steady-state growth in these *lacZssrA* strains **(Table S1)**, no significant growth rate differences were observed in minimal lactose media or other conditions **(Fig. 1A and Table S1)**. We quantified lag phase duration of each strain in glucose-to-lactose transitions, and found that strains with the lowest β-galactosidase expression levels (*NYNY* and *GSNY*) had the longest lag phase durations **(Fig. 1B)**. We therefore predicted that selection would not act on gene expression levels in a constant lactose environment, but could act against lower expression in fluctuating

glucose-lactose conditions due to the presence of increased lag phases. To test this hypothesis, we propagated the mixture of *lacZssrA* strains as a single batch culture in minimal media supplemented with either glucose or lactose as the only carbon source. Cultures were grown in either constant conditions or daily fluctuations between the two carbon sources, and samples were assayed at regular intervals to determine relative strain frequencies **(Fig. 1C)**. We initially ran a short pilot experiment lasting 5 days, and found that cultures grown in daily fluctuations between glucose and lactose exhibited selection against strains with the lowest expression levels, while cultures grown in constant conditions showed no significant fitness differences among the strains **(Fig. S1)**. We then repeated the experiment, expanding its duration to 14 days, and running separate experiments at three different daily dilution factors **(Figs. 1D, S2-S4)**. In each case, the experiments revealed that daily fluctuations between glucose and lactose select for higher *lac* expression levels. Measurement of selection coefficients revealed small (<1%), sustained and statistically significant fitness differences among the strains in the fluctuating environment **(Fig. 1E)**. Modeling of expression levels and induction kinetics recapitulated the overall small magnitude of selection coefficients **(Fig. S5)**, and predicted that the strength of selection for memory decreases with number of generations spent in each environment **(Fig. 1F)**. Consistent with the model, the highest selection coefficients were measured for the *No Deg* strain in the 1:16 daily cell dilution corresponding to the highest level of memory and the lowest number of generations in each condition **(Fig. 1F)**. Due to the larger number of generations in experiments with stronger dilution factors, beneficial mutations unrelated to *lac* expression were found to increase in frequency in the second half of several experiments **(Figs. S2-S4 and supplementary text)**. In such cases, selection coefficients for *lac* expression levels were inferred from the initial part of the experiment up to the point at which a selective sweep was observed to begin. Together, these results support our hypothesis that selection favors higher β-galactosidase expression levels in fluctuating environments, increasing the duration of phenotypic memory, and reducing lag phase durations.

Costs and benefits of gene regulation

Taking the above to its logical extreme, memory of arbitrary duration – corresponding to constitutive expression or the absence of gene regulation – is expected to eliminate lag phases altogether. Identifying fluctuating conditions that select against constitutive expression is therefore critical for understanding how gene regulation is maintained in nature. To this end, we deleted the *lacI* gene (*lacI*$^-$) in the *E. coli* chromosome to yield *lac* constitutive expression and tested its effect on expression, doubling time, and bioenergetics in various growth conditions **(Figs. 2, S6-S7, and**

Tables S2-S3). We measured the growth rates of the WT and *lacI*$^-$ strains in repeated glucose to lactose transitions using microfluidics **(Fig. 2A; Movies S1-S2)**. Unlike the WT, the *lacI*$^-$ strain grew continuously through the first transition to lactose without going through a lag phase. To assess the cost of constitutive expression, we grew cells in a panel of carbon sources, and quantified growth rates and β-galactosidase expression levels. Despite the substantial increase in *lac* expression levels resulting from increasing levels of cAMP as intracellular glucose levels decline in poorer media [3], there were no detectible costs for constitutive over-expression of the *lac* operon in any single carbon source that we tested **(Figs. 2B-C, S6)**. It was previously shown that the primary origin of fitness cost of *lac* expression in engineered *lac* operon strains was the activity of the lactose permease which uses the proton motive force (PMF) to transport lactose against a concentration gradient [13, 15, 30]. To directly quantify these costs in the native *lac* operon and in a range of *lac* expression levels, we compared growth rates of WT and *lacI*$^-$ strains in minimal media with and without a non-metabolizable inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG). We found highly significant decrease in growth rate, depending on the carbon source, confirming that the primary source of *lac* operon expression cost is the activity of LacY **(Figs. 2C, S6)**. The differences in growth rate and *lac* expression levels revealed a dependency in which transport-mediated growth rate cost increased with increasing *lac* expression levels **(Fig. S6)**. For example, in glycerol, which showed high *lac* expression **(Fig. 2B)**, we also observed a large growth rate cost (14%) upon addition of IPTG for both WT and *lacI*$^-$ strains **(Fig. 2C)**. In contrast, in glucose, when *lac* expression levels were the lowest, we observed the lowest growth rate cost (6%). To demonstrate that aerobic respiration was compromised due to LacY activity, we performed bioenergetic analysis and measured the oxygen consumption rate (OCR) for both WT and *lacI*$^-$ strains during growth in glucose or glycerol media supplemented with and without IPTG **(Fig. 2D)**. We found statistically significant differences in OCR with and without IPTG in all tested conditions **(Fig. 2D)**. The difference in OCR with and without IPTG was significantly more pronounced in glycerol (19%) than in glucose (11%) **(Fig. 2D)**, consistent with the larger growth rate costs measured in glycerol **(Fig. 2C)**. These data confirm a long-standing hypothesis [15, 31, 32] in which LacY activity that depletes the PMF consequently leads to a decrease in aerobic respiration. Collectively, our results establish that constitutive expression of Lac proteins on its own does not incur costs, but transport of a non-metabolizable molecule through LacY, which diffuses the PMF and directly affects aerobic respiration, reduces growth rates proportionally to operon expression level.

Based on these findings, we hypothesized that growth costs will result if cells transport more

lactose than necessary to support growth on lactose, e.g. if the *lac* permease is over-expressed. We therefore investigated more complex growth transitions involving diauxic growth, in which cells are grown on a mixture of two different carbon sources [33-41]. We grew WT and *lacI*$^-$ strains in one of seven different carbon sources and then transferred cells to a mixture of the same carbon source and lactose. In diauxic growth on glucose and lactose, WT displayed classic diauxic growth wherein cells first consume glucose and then go through a diauxic lag phase before continuing to grow in lactose **(Figs. 3A-B, S8)**. The *lacI*$^-$ strain, however, grew continuously without a lag phase, indicating that constitutive *lac* expression is beneficial in these conditions. Additionally, the *lacI*$^-$ strain exhibited faster growth in lactose diauxic conditions with arabinose, xylose, or maltose **(Fig. S9)**. In contrast, we observed a prolonged lag and recovery phase for the *lacI*$^-$ strain in glycerol and lactose diauxic growth while the WT avoided a lag phase and exhibited fast growth at the outset **(Figs. 3C-D, S10)**. Similar results were obtained in lactose diauxie with pyruvate or succinate **(Fig. S11)**. We propose that this 'over-expression lag' is caused by expression of LacY above a threshold prior to transfer into diauxic conditions. This results in growth rate reduction of the *lacI*$^-$ strain due to the excessive flux of lactose into the cell which is deleterious to the PMF and aerobic respiration, while WT avoids this cost by allowing cAMP levels to decrease gradually during *lac* operon induction which prevents *lac* over-expression.

Selection for gene regulation

Since the growth lags observed above would be detrimental to long-term growth, we hypothesized that *lac* regulation could be selected for or against under fluctuating diauxie, in which lactose is periodically presented together with a second carbon source. We designed competition-based experiments to test this hypothesis, in which we co-cultured WT, *lacI*$^-$, and *NYNY* strains and subjected these to daily fluctuations between growth in a single carbon source (glucose or glycerol) and diauxic growth conditions with lactose (see Methods). In periodic glucose-lactose diauxie, we observed a continuous increase of the *lacI*$^-$ strain frequency while WT remained unchanged and *NYNY* decreased from its starting frequency **(Fig. 3E)**. This demonstrates the benefit of constitutive *lac* expression in these conditions, and additionally, the gradual decrease of *NYNY* recapitulates our previous observation regarding the benefit of phenotypic memory in glucose-lactose fluctuations (compare **Fig. 1B**). Strikingly, in periodic glycerol-lactose diauxie, we observed extinction of the *lacI*$^-$ strain by day 12 **(Fig. 3F)**, while the WT strain frequency nearly doubled over the course of the experiment. In these conditions, *NYNY* initially increased relative to *lacI*$^-$ but once the frequency of *lacI*$^-$ had decayed, *NYNY* began to gradually decrease relative to WT **(Fig. 3F)**. In both control conditions, all three strains were maintained at similar frequencies

throughout the experiment **(Fig. S12)**. These results establish that fluctuating lactose diauxie using a poor carbon source selects for gene regulation in the *lac* operon, while simultaneously selecting against lower *lac* expression levels that decrease phenotypic memory.

Sign epistasis between sensing and control of expression in a gene regulatory network

To investigate how presence or absence of sensing interacts with genetic mutations that control expression levels, in both WT and *lacI*$^-$ strains we edited the UP element (-45 region) of the *lac* promoter, which impacts Crp-dependent activation [42-45], generating a library of strains with altered *lac* expression levels **(Table S4)**. In glucose and lactose diauxie, the WT$_{UE}$ strain library exhibited a range of diauxic lag phases that increased as *lac* expression decreased **(Figs. 4A, S13A)**. In comparison, the *lacI*$^-_{UE}$ library maintained the benefits of constitutive *lac* expression by eliminating the glucose to lactose diauxic lag phase despite expression being reduced by up to 49% **(Figs. 4B, S13B)**. We then measured the growth in glycerol and lactose diauxie which showed substantial growth cost for constitutive *lac* expression **(Figs. 4C-D, S13C-D)**. In particular, the *lacI*$^-_{UE}$ library exhibited much wider lag time variability compared to WT$_{UE}$ strains **(Figs. S13E-H)**. Lag time variation among the WT$_{UE}$ strains was relatively small, which supports our previous findings that *lac* negative regulation prevents growth cost in these conditions **(Figs. 4C, S13C)**. Importantly, the *lac* repressor acts to buffer the genetic variation revealed in the *lacI*$^-_{UE}$ library. Not only does the repressor reduce variation in lag durations which impact fitness, its presence changes the sign of fitness differences (compare order of curves in **Figs. 4C-D**). This genetic interaction, known as sign epistasis [18], between mutations impacting sensing and control of expression, may enhance the ability of gene regulatory networks to adapt as discussed below.

Discussion

Our investigation provides a systematic basis for understanding the selective constraints that shape the two main functions of a gene regulatory circuit, environmental sensing and control of expression. Our first set of experiments perturbed protein expression levels using degradation tags, without altering regulators or their binding sites. These experiments revealed that reduction of β-galactosidase expression by 30% did not affect the ability of cells to grow on lactose, but could be selected against in a fluctuation environment due to its reduction of phenotypic memory and increase of lag duration in glucose-lactose transitions **(Fig. 1)**. Next, we eliminated negative regulation by deleting the *lac* repressor and compared constitutive expression with wild-type

regulation. We found that constitutive expression of the *lac* operon did not incur costs in any of the tested carbon sources, despite expression levels varying up to 5 times higher than the maximal expression level in lactose **(Figs. 2C, S6, and Table S2)**. Instead, we found that costs manifest only in fluctuating diauxic conditions when *lac* expression levels cross a threshold. Above a threshold expression level, which we estimate to be ~3 times the normal expression levels in lactose, the lag phase upon transition to lactose diauxie becomes substantially longer than the cell division time in constant environments **(Figs. 3A-D, S9-S11)** These results are consistent with a model in which the molecular cause of this 'over-expression lag' stems from the wasteful flux of lactose through the *lac* permease, which degrades the proton gradient and reduces the capacity for cells to produce ATP through aerobic respiration **(Figs. 2C-D)**. In order to exit this lag, *lacI*$^-$ cells could metabolize the incoming lactose into glucose, which enables slow growth before the PMF has recovered [46], and causes cAMP levels to decrease, gradually reducing *lac* expression levels. WT cells have an advantage over *lacI*$^-$ in such conditions because presence of the repressor enables expression levels to increase gradually, which concomitantly decreases cAMP levels due to increasing activity of β-galactosidase metabolizing lactose. Competition experiments demonstrate that fluctuating lactose selects for sensing regulation (WT) in a high cAMP context such as glycerol **(Fig. 3F)**, or for constitutive expression (*lacI*$^-$) in a low cAMP context such as glucose **(Fig. 3E)**.

By sensing and responding to environmental fluctuations, the *lac* repressor shapes the dynamics of gene expression to avoid over-expression lags. In doing so, the repressor also plays an important role in shaping the environment-dependent fitness landscape in which gene expression levels adapt. In the absence of the repressor, glucose-lactose diauxic conditions would select for increasing expression levels **(Fig. 4B)**, while glycerol-lactose diauxie select for decreasing expression **(Fig. 4D)**. Such conflicting selection pressures reduce both the efficiency of selection and the maximal long-term growth rate in fluctuating environments. Presence of the repressor enables directional adaptation of gene expression levels across conditions (compare order of curves in **Figs. 4A,C** versus **4B,D**). Additionally, we observed environment-dependent sign epistasis between mutations impacting sensing (WT vs. *lacI*$^-$) and control of expression levels (*UE* strains) in the glycerol-lactose diauxic condition **(Figs. 4C-D)**. Due to sign epistasis, as gene expression levels adapt toward higher levels in the presence of the repressor **(Figs. 4A-C)**, the fitness cost of *lacI*$^-$ mutations during glycerol-lactose diauxie increases **(Fig. 4D)**, making it increasingly difficult to lose the repressor when such environments recur. Nevertheless, presence of the repressor incurs a short lag phase (~1 generation) each time the operon must be induced.

Phenotypic memory, resulting from inherited *lac* proteins, mitigates this effect by eliminating or reducing lag phase durations in a fluctuating environment. The competition experiments related to phenotypic memory revealed that small selection coefficients (<1%) shape the evolution of gene expression levels in a fluctuating environment **(Figs. 1D-E)**, and that such adaptation is possible in fluctuating conditions that maintain the repressor **(Fig. 3F)**.

In their natural setting, *E. coli* cells reside in the gastrointestinal tract of mammals where they participate in interspecies interactions within the gut microbiome, which includes competition for resources [47]. It was shown that in the murine gut, *E. coli* WT and *lacI*$^-$ strains coexist in lactose diet conditions, while experiments *in vitro* showed that *lacI*$^-$ had shorter lag times in glucose-to-lactose transitions [48]. Additionally, the competitive advantage of the *lac* operon is conditional on lactose being present in the mouse diet and on the presence of a diverse microbiome [49]. In light of these findings, our work suggests that periods of glucose starvation, in which bacteria utilize poor carbon sources, combined with transient availability of lactose, are common features of the host niche, which would select for maintenance of the regulator.

Our results point to a previously unexplored role of gene regulation in determining how gene expression levels evolve in fluctuating environments, and suggest that the cost-benefit tradeoffs found here may exist in other gene regulatory networks with similar properties. By uncovering the connections between the character of the fluctuating environment, the molecular basis of expression costs, and the role of gene regulation in shaping an evolutionary landscape, our work provides a new basis for understanding and predicting how gene regulatory networks may evolve.

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Author contributions

D.P. and E.K. designed research, performed research, analyzed data, and wrote the paper; T.N. analyzed data.

References

1. Wadhams, G.H. and J.P. Armitage, *Making sense of it all: bacterial chemotaxis.* Nat Rev Mol Cell Biol, 2004. **5**(12): p. 1024-37.

2. Aguilar, C., et al., *Thinking about Bacillus subtilis as a multicellular organism.* Curr Opin Microbiol, 2007. **10**(6): p. 638-43.

3. Gorke, B. and J. Stulke, *Carbon catabolite repression in bacteria: many ways to make the most out of nutrients.* Nat Rev Microbiol, 2008. **6**(8): p. 613-24.

4. Bervoets, I. and D. Charlier, *Diversity, versatility and complexity of bacterial gene regulation mechanisms: opportunities and drawbacks for applications in synthetic biology.* FEMS Microbiol Rev, 2019. **43**(3): p. 304-339.

5. Carthew, R.W., *Gene Regulation and Cellular Metabolism: An Essential Partnership.* Trends Genet, 2021. **37**(4): p. 389-400.

6. McAdams, H.H., B. Srinivasan, and A.P. Arkin, *The evolution of genetic regulatory systems in bacteria.* Nat Rev Genet, 2004. **5**(3): p. 169-78.

7. Perez, J.C. and E.A. Groisman, *Evolution of transcriptional regulatory circuits in bacteria.* Cell, 2009. **138**(2): p. 233-44.

8. Quan, S., et al., *Adaptive evolution of the lactose utilization network in experimentally evolved populations of Escherichia coli.* PLoS Genet, 2012. **8**(1): p. e1002444.

9. Kvitek, D.J. and G. Sherlock, *Whole genome, whole population sequencing reveals that loss of signaling networks is the major adaptive strategy in a constant environment.* PLoS Genet, 2013. **9**(11): p. e1003972.

10. Van den Bergh, B., et al., *Experimental Design, Population Dynamics, and Diversity in Microbial Experimental Evolution.* Microbiol Mol Biol Rev, 2018. **82**(3).

11. Ghalayini, M., et al., *Long-term evolution of the natural isolate of Escherichia coli 536 in the mouse gut colonized after maternal transmission reveals convergence in the constitutive expression of the lactose operon.* Mol Ecol, 2019. **28**(19): p. 4470-4485.

12. Cooper, T.F. and R.E. Lenski, *Experimental evolution with E. coli in diverse resource environments. I. Fluctuating environments promote divergence of replicate populations.* BMC Evol Biol, 2010. **10**: p. 11.

13. Dekel, E. and U. Alon, *Optimality and evolutionary tuning of the expression level of a protein.* Nature, 2005. **436**(7050): p. 588-92.

14. Scott, M., et al., *Interdependence of cell growth and gene expression: origins and consequences.* Science, 2010. **330**(6007): p. 1099-102.

15. Eames, M. and T. Kortemme, *Cost-benefit tradeoffs in engineered lac operons.* Science, 2012. **336**(6083): p. 911-5.

16. Bertrand, R.L., *Lag Phase Is a Dynamic, Organized, Adaptive, and Evolvable Period That Prepares Bacteria for Cell Division.* J Bacteriol, 2019. **201**(7).

17. Phillips, K.N., et al., *Diversity in lac Operon Regulation among Diverse Escherichia coli Isolates Depends on the Broader Genetic Background but Is Not Explained by Genetic Relatedness.* mBio, 2019. **10**(6).

18. Taute, K.M., et al., *Evolutionary constraints in variable environments, from proteins to networks.* Trends Genet, 2014. **30**(5): p. 192-8.

19. Karasov, T.L., et al., *Mechanisms to Mitigate the Trade-Off between Growth and Defense.* Plant Cell, 2017. **29**(4): p. 666-680.

20. Casadesus, J. and R. D'Ari, *Memory in bacteria and phage.* Bioessays, 2002. **24**(6): p. 512-8.

21. Stockwell, S.R., C.R. Landry, and S.A. Rifkin, *The yeast galactose network as a quantitative model for cellular memory.* Mol Biosyst, 2015. **11**(1): p. 28-37.

22. Bleuven, C. and C.R. Landry, *Molecular and cellular bases of adaptation to a changing environment in microorganisms.* Proc Biol Sci, 2016. **283**(1841).

23. Vermeersch, L., et al., *Do microbes have a memory? History-dependent behavior in the adaptation to variable environments.* Front Microbiol, 2022. **13**: p. 1004488.

24. Lambert, G. and E. Kussell, *Memory and fitness optimization of bacteria under fluctuating environments.* PLoS Genet, 2014. **10**(9): p. e1004556.

25. Poelwijk, F.J., M.G. de Vos, and S.J. Tans, *Tradeoffs and optimality in the evolution of gene regulation.* Cell, 2011. **146**(3): p. 462-70.

26. Poelwijk, F.J., et al., *Optimality and evolution of transcriptionally regulated gene expression.* BMC Syst Biol, 2011. **5**: p. 128.

27. Sinha, A.K., M.F. Laursen, and T.R. Licht, *Regulation of microbial gene expression: the key to understanding our gut microbiome.* Trends Microbiol, 2025. **33**(4): p. 397-407.

28. Nielsen, J. and J.D. Keasling, *Engineering Cellular Metabolism.* Cell, 2016. **164**(6): p. 1185-1197.

29. Pollack, D., T. Nozoe, and E. Kussell, *Proteolytic stability and aggregation in a key metabolic enzyme of bacteria.* Proc Natl Acad Sci U S A, 2024. **121**(19): p. e2301458121.

30. Stoebel, D.M., A.M. Dean, and D.E. Dykhuizen, *The cost of expression of Escherichia coli lac operon proteins is in the process, not in the products.* Genetics, 2008. **178**(3): p. 1653-60.

31. Dykhuizen, D. and D. Hartl, *Transport by the lactose permease of Escherichia coli as the basis of lactose killing.* J Bacteriol, 1978. **135**(3): p. 876-82.

32. Moreno-Gamez, S., et al., *Wide lag time distributions break a trade-off between reproduction and survival in bacteria.* Proc Natl Acad Sci U S A, 2020. **117**(31): p. 18729-18736.

33. Monod, J., *The Growth of Bacterial Cultures.* Annual Review of Microbiology, 1949. **3**: p. 371-394.

34. Loomis, W.F., Jr. and B. Magasanik, *Glucose-lactose diauxie in Escherichia coli.* J Bacteriol, 1967. **93**(4): p. 1397-401.

35. Lendenmann, U., M. Snozzi, and T. Egli, *Kinetics of the simultaneous utilization of sugar mixtures by Escherichia coli in continuous culture.* Appl Environ Microbiol, 1996. **62**(5): p. 1493-9.

36. Inada, T., K. Kimata, and H. Aiba, *Mechanism responsible for glucose-lactose diauxie in Escherichia coli: challenge to the cAMP model.* Genes Cells, 1996. **1**(3): p. 293-301.

37. Boulineau, S., et al., *Single-cell dynamics reveals sustained growth during diauxic shifts.* PLoS One, 2013. **8**(4): p. e61686.

38. Solopova, A., et al., *Bet-hedging during bacterial diauxic shift.* Proc Natl Acad Sci U S A, 2014. **111**(20): p. 7427-32.

39. Aidelberg, G., et al., *Hierarchy of non-glucose sugars in Escherichia coli.* BMC Syst Biol, 2014. **8**: p. 133.

40. Wang, X., et al., *Growth strategy of microbes on mixed carbon sources.* Nat Commun, 2019. **10**(1): p. 1279.

41. Okano, H., et al., *Regulation underlying hierarchical and simultaneous utilization of carbon substrates by flux sensors in Escherichia coli.* Nat Microbiol, 2020. **5**(1): p. 206-215.

42. Czarniecki, D., R.J. Noel, Jr., and W.S. Reznikoff, *The -45 region of the Escherichia coli lac promoter: CAP-dependent and CAP-independent transcription.* J Bacteriol, 1997. **179**(2): p. 423-9.

43. Estrem, S.T., et al., *Bacterial promoter architecture: subsite structure of UP elements and interactions with the carboxy-terminal domain of the RNA polymerase alpha subunit.* Genes Dev, 1999. **13**(16): p. 2134-47.

44. Flatow, U., G.V. Rajendrakumar, and S. Garges, *Analysis of the spacer DNA between the cyclic AMP receptor protein binding site and the lac promoter.* J Bacteriol, 1996. **178**(8): p. 2436-9.

45. Noel, R.J., Jr. and W.S. Reznikoff, *CAP, the -45 region, and RNA polymerase: three partners in transcription initiation at lacP1 in Escherichia coli.* J Mol Biol, 1998. **282**(3): p. 495-504.

46. Kinoshita, N., T. Unemoto, and H. Kobayashi, *Proton motive force is not obligatory for growth of Escherichia coli.* J Bacteriol, 1984. **160**(3): p. 1074-7.

47. Kern, L., et al., *Commensal inter-bacterial interactions shaping the microbiota.* Curr Opin Microbiol, 2021. **63**: p. 158-171.

48. Cherrak, Y., et al., *Non-canonical start codons confer context-dependent advantages in carbohydrate utilization for commensal E. coli in the murine gut.* Nat Microbiol, 2024. **9**(10): p. 2696-2709.

49. Pinto, C., et al., *The Selective Advantage of the lac Operon for Escherichia coli Is Conditional on Diet and Microbiota Composition.* Front Microbiol, 2021. **12**: p. 709259.

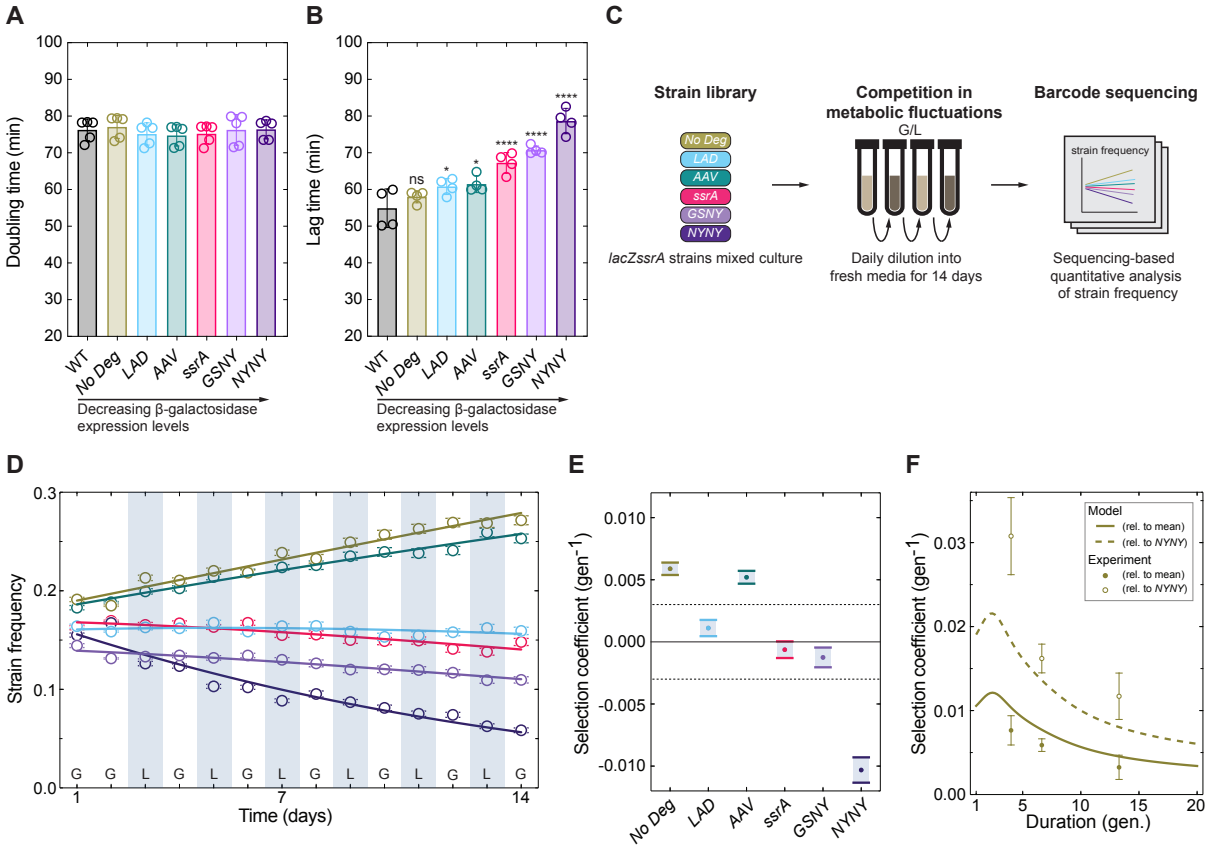

Fig. 1. Selection for phenotypic memory in a fluctuating environment. (A) Doubling time measured for WT *E. coli* and *lacZssrA* strains grown in lactose minimal media. Strains are ordered according to β-galactosidase expression levels during induction in lactose. Bars and error bars indicate mean ± std. dev. (SD) over biological replicates (open circles, n = 5); one-way ANOVA, *p*-value = 0.89. See also Table S1. **(B)** Duration of lag phase upon glucose-to-lactose transition. One-way ANOVA with Dunnett multiple comparisons test of each strain to the WT was used for statistical analysis. Statistically significant differences are indicated (****p<1e-4, *p<0.05, ns = p>0.05). **(C)** Schematic of mixed-culture competition assay. The library of *lacZssrA* strains in equal ratios was inoculated at t = 1 and propagated in 9 parallel experiments for 14 days. Strains were sampled and diluted (1:16, 1:100, or 1:10,000) into fresh media alternating glucose/lactose (G/L) every 24 h. Controls were grown for the same duration and dilutions in either constant glucose or lactose media. DNA was extracted from each sample and the 3' end of the *lacZ* gene was amplified by PCR. Pooled samples were sequenced and read counts were used to determine strain frequencies. **(D)** Frequency of *lacZssrA* strains over two weeks grown in alternating G/L with 1:100 daily dilution. Relative exponential growth model was fit (solid lines) to infer selection coefficients. Error bars indicate ± standard error of the mean (SEM) based on a multinomial distribution of read counts. **(E)** Relative selection coefficients (points) and 95% confidence intervals (bars) are shown for each strain inferred from model fitting in Fig. 1D. Dashed lines indicate upper and lower bound of coefficient confidence intervals inferred from competition in constant glucose. See also Figs. S1-S4 and supplementary text for further details. **(F)** Predicted dependence of relative selection coefficients on number of generations between environmental changes. Model predictions (curves) and measurements (points) of selection coefficients are shown for the *No Deg* strain relative to either the *NYNY* strain or the mean over all 6 strains. See also Fig. S5.

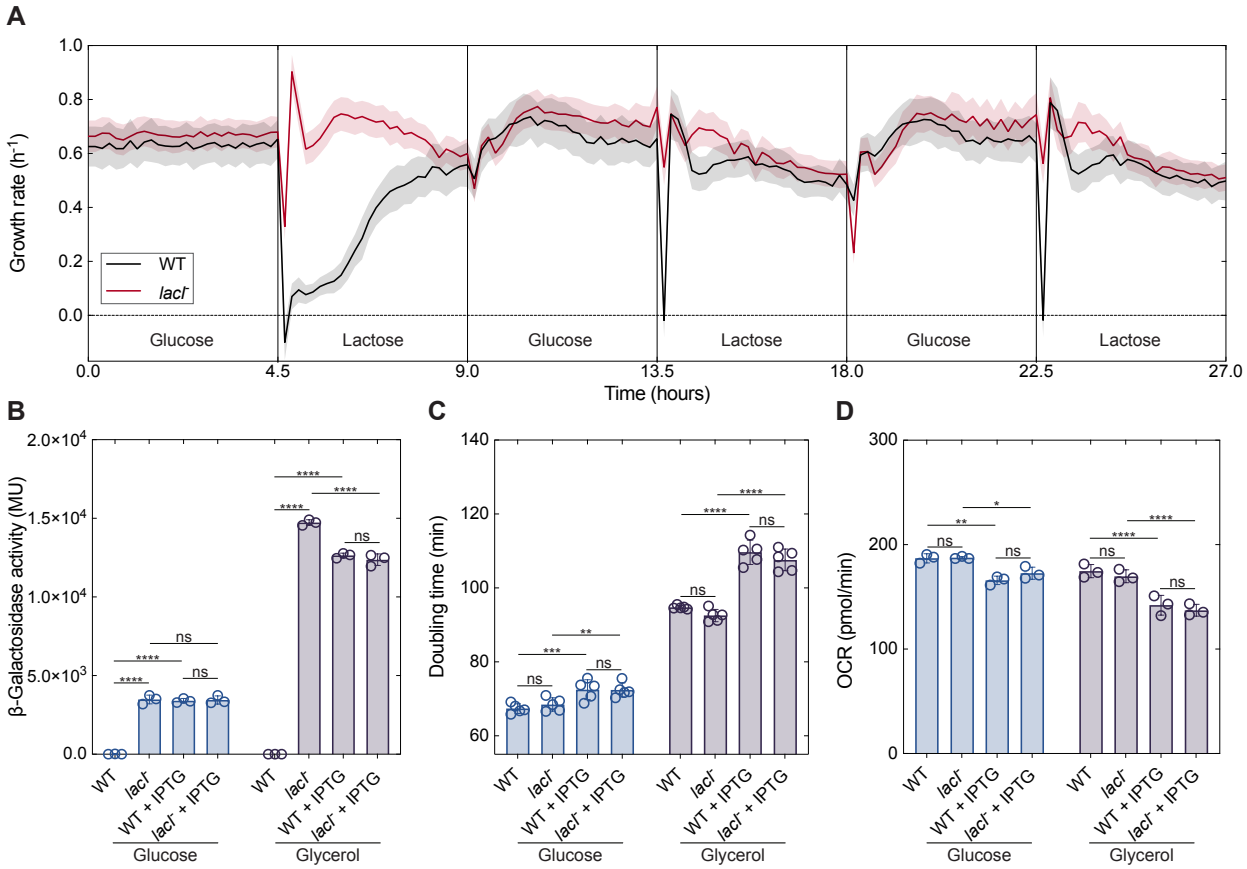

Fig. 2. Lag phase dynamics and cost of expression with and without gene regulation.
(A) Growth rate measurements of WT and *lacI*$^-$ strains in periodic glucose-lactose fluctuations using microfluidics. Cells were grown in minimal media with lactose or glucose as carbon source, and supplemented with 1% casamino acids. Mean growth rate of cells in microfluidic chambers is shown (solid line) ± SEM (shaded envelope). See also movies S1-S2. **(B)** β-Galactosidase activity levels in Miller units (MU) are shown for WT and *lacI*$^-$ strains grown in minimal media supplemented with glucose or glycerol, with or without 1mM IPTG. Bars indicate mean ± SD (n = 3). See also Fig. S6. **(C)** Doubling time measured for WT and *lacI*$^-$ strains in the same conditions as panel B. Individual biological replicates (n = 5) are shown in circles. Growth rate cost for each strain was calculated by [μ(+IPTG) – μ(-IPTG)] / μ(-IPTG) where μ is measured growth rate. See also Fig. S6. **(D)** Oxygen consumption rate (OCR, in picomoles of molecular oxygen per minute) is shown for WT and *lacI*$^-$ cultures grown in minimal media supplemented with glucose or glycerol, with or without IPTG. Measurements were acquired using a Seahorse XF Pro Flux Analyzer. OCR data is shown for averages of t = 0 – 10 minutes ± SD (n = 3). Two-way ANOVA with Tukey multiple comparisons test was used for statistical analysis in panels B-D. Statistically significant differences are indicated (****p<1e-4, ***p<1e-3, **p<1e-2, *p<0.05, ns = p>0.05).

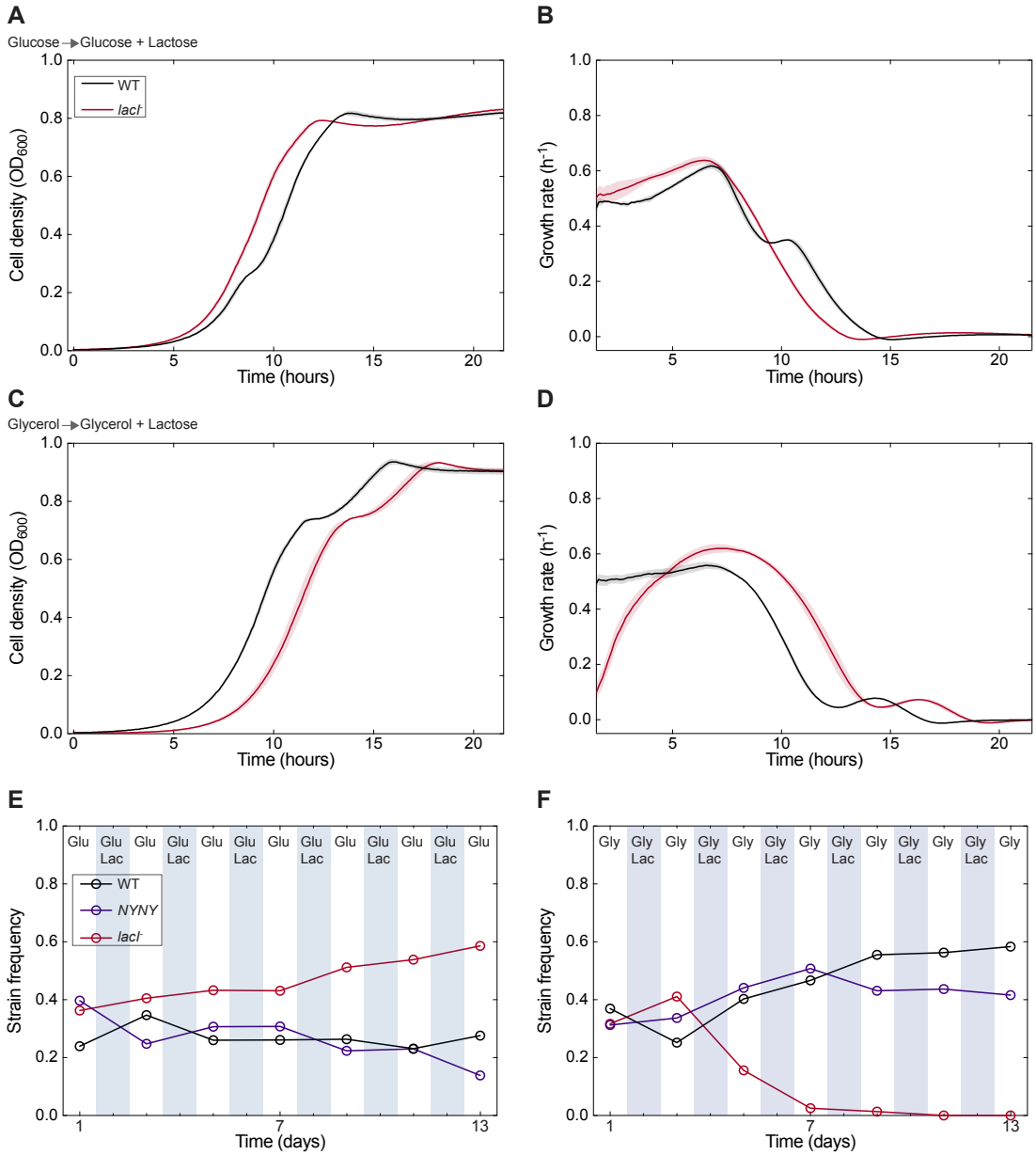

Fig. 3. Selection for or against gene regulation in fluctuating diauxic conditions.
(A-D) Diauxic growth dynamics of WT and *lacI*$^-$ strains. OD_{600} (A and C) and instantaneous growth rates (B and D) shown as mean (solid line) ± SEM (shaded envelope) over biological replicates (n = 3). (A and B) WT and *lacI*$^-$ strains grown separately in mixture (1:2 v/v) of glucose and lactose minimal media. Cells were grown overnight in glucose and diluted to an OD_{600} = 0.01 at t = 0. See also Fig. S8 and S9. (C and D) WT and *lacI*$^-$ grown separately in mixture (1:2 v/v) of glycerol and lactose minimal media. Cells were grown overnight in glycerol and diluted to an OD_{600} = 0.01 at t = 0. See also Fig. S10 and S11. **(E-F)** Competition experiments in diauxic fluctuating environments. A mixed culture of WT, *lacI*$^-$, and *NYNY* strains combined in equal ratios at t = 1 based on normalized OD_{600} was grown in a periodic environment alternating between glucose-only media and glucose and lactose, shown in panel E; or between glycerol-only media and glycerol and lactose diauxie, shown in panel F. See also Fig. S12.

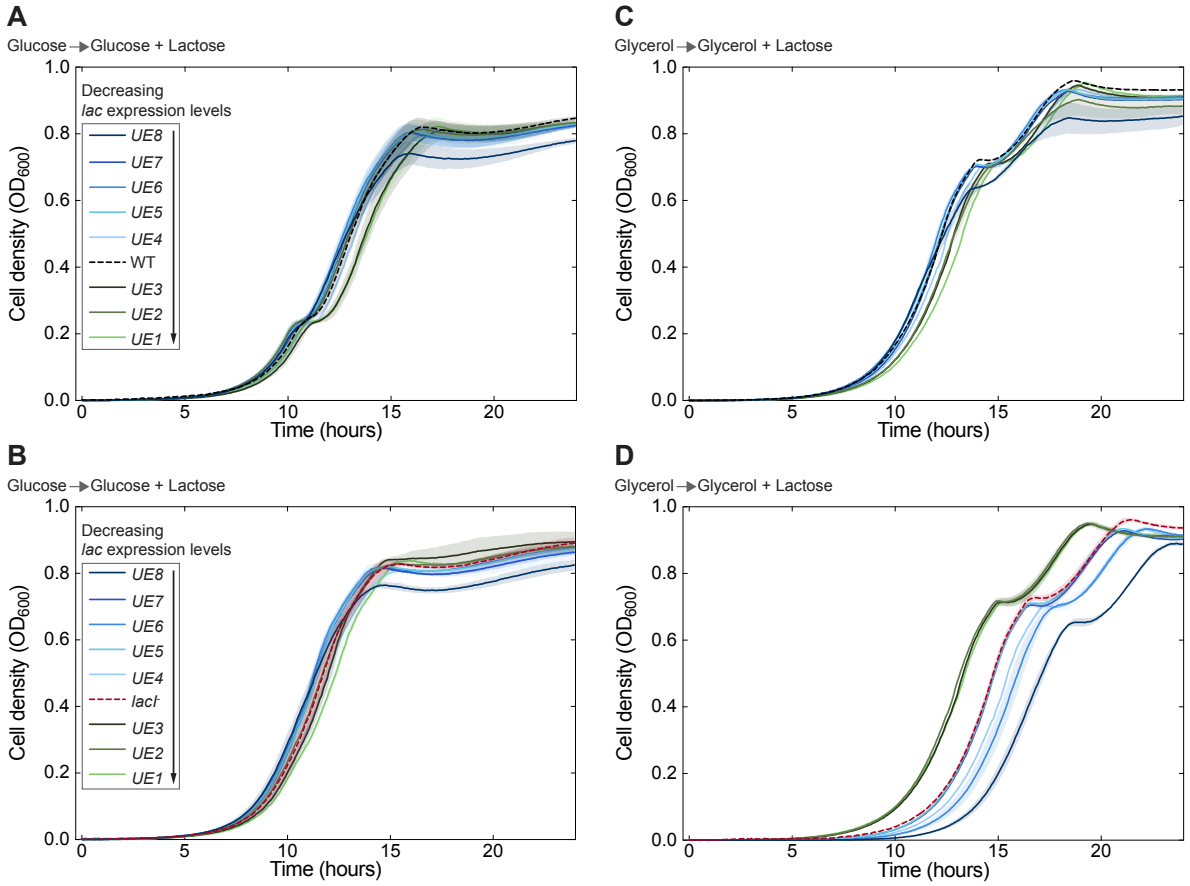

Fig. 4. Epistatic interaction between sensing and control of gene expression.
Diauxic growth dynamics of the UE strain library in WT **(A and C)** and *lacI*$^-$ **(B and D)** backgrounds. Each strain was grown separately in a mixture (1:2 v/v) of glucose **(A and B)** or glycerol (**C and D)** and lactose minimal media. Cells were grown overnight in glucose **(A and B)** or glycerol **(C and D)** and diluted to an OD_{600} = 0.01 at t = 0. Legend indicates strains ordered by *lac* operon expression levels. Curves show mean OD_{600} measurements taken every 5 minutes over biological replicates (n = 3); ± SEM is shown in shaded envelope. See also Fig. S13 and Table S4.