Lenski's Long Term *E.coli* Experiment: A Literature Review of Parallel Mutations Contributing the Most to Fitness Gains

Gene	Function	Parallel	Gen. Fixed	Resulting Phenotype/Trade-offs	Fitness Gain
nadR	Encodes repressor of the NAD biosynthesis genes.[3]	12 of 12[1,2]		"The IS*150* -E insertion almost certainly inactivated *nadR* ."[3] | "The evolved lines have higher maximum growth rates than their ancestor, and these fast growing cells may need more NAD than do cells growing slowly, suggesting a possible advantage for this mutation."[3] | "High NAD+/NADP+ levels may promote repair of oxidized proteins in starving cells."[3]	8.1%[1]
pykF	Core regulatory enzyme in glycolysis metabolism. | "…the *pykF* gene which encodes pyruvate kinase I, one of two glycolitic isoenzymes that catalyze the conversion of phosphoenolypyruvate (PEP) and ADP into pyruvate and ATP."[3]	12 of 12[1,2]	5k -> Fixed[1]	"The insertion almost certainly inactivated *pykF* ."[3] | "…PEP is the proximate source of phosphate for enzyme I in the phosphotransferase system (PTS), by which glucose and certain other substrates are transported across the inner membrane of E. coli…. Viewed in this light, the pykF:IS*150* insertion may provide a benefit by slowing the conversion of PEP to pyruvate, such that more PEP is available to drive the PTS."[3] (More PTS means more glucose transport into the cell).	11.1%[1]
rbs op.	Involved in making the sugar ribose.	12 of 12[1]	Fixed by 2k[4]	"Twelve populations of Escherichia coli B all lost D-ribose catabolic function during 2,000 generations…"[4] | "…involved the deletion of part or all of the ribose operon (*rbs* genes)."[4]	2.1%[4]
malT op.	Involved in the metabolism of maltose and maltodextrins.	8 of 12[6]	5k -> Fixed[1]	"On a whole, these experiments showed reduction in the activity of the maltose regulon consistent with the predicted loss of function of the MalT transcriptional activator"[6]	.4%[6]
spoT	"Encodes a protein involved in synthesis and degredation of ppGpp, the moleculor effector of the stringent response which is the global regulatory network involved in adaptation to nutritional stress."[5]	8 of 12[6]	2k-> Fixed[1]	"First, the array data show that the *spoT* mutation lowers expression of the flagella-encoding *flg* operons. The ancestral strain used in the evolution experiment was nonmotile, the selective environment lacked physical structure, and the production of flagella is known to be costly. Hence, reducing the expression of these genes could be beneficial. Second, a reduction in the concentration of ppGpp, shown to result from mutations in the regulatory region of *spoT* , might increase the rate of transcritpion from tRNA and rRNA promoters. This increased transcription raises the maximal growth rate, presumably via an increased speed of translation during growth in minimal medium."[5]	9.4%[5] †
pbpA op.Ŧ	The *pbpA* operon encodes the PBP2 (penicillin binding protein 2) and *RodA* proteins which are involved in cell wall bio-	6 of 12[9]	2k-> Fixed[9] 5k -> Fixed[1]	"Evolved *pbpA* alleles reduce the expression of PBP2."[9] | "Both evolved mutations in the *pbpA* promoter region caused substantial reductions in the transcription of the operon."[9] | "The evolved *pbpA* mutations also had plieotropic effects that were detrimental under certain other conditions. In particular, both evolved pbpA alleles caused increased sensitivity to	

	synthesis and determination of cell shape.[9,1] | "The *pbpA* gene encodes penicillin-binding protein 2 which is involved in peptidoglycan synthesis and elongation of the cell wall; the characteristic rod shape of E. coli cells also depends on the rodA gene."[3]			osmotic stress. We also showed that the evolved *pbpA* -5 allele reduced competitive performance during prolonged starvation over many days…"[9] | PBP2 inhibition has been reported to "delay the initation of both peptoglycan synthesis and growth when cells are in stationary phase."[9,10] | "As a consequence of the increased size and altered shape, the average ratio of surface area to volume declined in the evolved bacteria relative to the ratio for their common ancestor."[9] | "Thus, both evolved pbpA alleles seem to disrupt cell wall elongation during exponential growth, as previously described for impairment of PBP2 activity."[9] | "…both mutations decreased cellular resistance to osmotic stress. Moreover, one mutation reduced fitness during prolonged stationary phase. Therefore, alteration of the PBP2 concentration contributed to physiological trade-offs… in performance in other environments."[9]	
topA *fis*	*TopA* and *fis* genes affect DNA topology- control supercoiling.[11] *TopA* encodes for topoisomerase I which relaxes DNA. *Fis* encodes the fis repressor protein that controls the level of DNA supercoiling.[11]	10 of 12[11] ₣	2k-Fixed[1,11]	"As shown in Figure 2, both the topA and fis mutations, when introduced separately into the ancestral chromosome, caused increases in DNA supercoiling."[11] | "…increased DNA superhelicity."[9] | "An increase in supercoiling would facilitate transcription of the rRNA operons. A higher rate of rRNA synthesis could be advantageous because the evolved lines have substantially higher exponential growth rates than does the ancestor. Cells growing faster may need more rRNA than cells growing slowly, and the ratio of RNA to DNA appears to have increased in the evolved lines."[11]‡‡ | "Fis represses transcription of gyrAB and reduces the activity of DNA gyrase, which may explain the increased level of supercoiling caused by the evolved fis allele."[11] | "These data also imply a reduction of the DNA relaxing activity of topoisomerase I in the evolved clones because this protein otherwise reduces supercoiling."[11] | "The evolved fis allele had somewhat less than half the ancestral level of *fis* protein, while the fis-deletion strain produced no detectable fis."[11] | "The *topA* mutation decreased the activity of the enzyme, while the *fis* mutation decreased the amount of fis gene product produced."[7]	13.3%[1,11] 2.9%[1,11]
citT op.	Encodes a protein that transports a range of citrate-like chemicals.	1 of 12[12]	> 31,500 - dominant allele, but not fixed.[12]	"The structure of the *cit* amplification (*rnk-citT* module) led us to propose that the Cit+ trait arose from an amplification-mediated promoter capture."[12] | "The native *citT* regulatory region showed no expression (above background) in any strain, indicating that *citT* is normally silent under oxic conditions…. These results indicate that the *rnk-citT* module can support *citT* expression during aerobic metabolism."[12] | To summarize, in the Ara-3 clone, a 2,933 bp duplication inserted into the *rnk* gene subverting it under the regulatory control of a different pre-existing promoter (the *rnk* promoter) that lacked an "oxygen off" switch. This rearrangement resulted in the constitutive expression the *citT* operon (the Cit+ phenotype) which was further refined due to uncontrolled amplification. | "Amplifications tend to be unstable."[12] | "There was	

				a several-fold increase in total population size owing to the high concentration of citrate relative to glucose in the medium."[12] | "These early Cit+ genomes also show increases in cit copy number. three-copy tandem array within a larger tandem duplication, a four copy tandem array, a tandem duplication in a larger three-copy tandem array. Changes in amplification copy number readily occur by recombination… These changes increased the number of rnk-citT modules relative to the earliest Cit+ genome…. The increased number of rnk-citT modules can thus explain the refinement of the Cit+ phenotype that allowed the population expansion."[12]	
mutT	"The MutT protein is a hydrolase that purges the celluar nucleotide pool of oxidized guanine nucleo-tides (8-oxo-dGTP), which can mis-pair with adenine and lead to A:T -> C:G (adenine or thymine to cytosine or guanine) trans-versions after DNA replication."[13]	1 of 12[13]	~20k - 25k[13]	"…the *mutT* mutation increased the point-mutation rate by ~150 fold…."[13] | "Moreover, hyper-mutators are more likely to produce offspring with deleterious or lethal mutations."[13] | "…the *mutT* lineage had a greatly elevated mutation rate, the increased mutations caused by *mutT* hypermutators are A:T -> C:G transversions."[13,14] | "The *mutT* mutant of E. coli is specifically elevated for the AT -> CG transversions and is one of the strongest mutators known, with a 10,000- to 50,000-fold incrase over the wildtype."[14]	
mutY	Encodes a DNA repair glycoslase that excises mis-paired bases.[13]	1 of 12[13]	~25,633k[13]	"Loss-of-function mutations in *mutY* , which encodes a DNA repair glycosylase that excises mis-paired bases from DNA helices, also lead ot elevated mutation rates…"[13] | "…*mutY* mutations have an antimutator effect in the context of a MutT defect because MutY mis-repairs 8-oxoG:A base pairs in DNA. The 60% reduction in overall mutation rates reported in *mutT mutY* double mutants compared with mutT single mutants is simlar to the rate changes we observed in both phylogenomic analysis (Fig. 1B) and fluctuation tests (Fig. 1C). Indeed, genome resequencing showed that different *mutY* mutations occurred along the two mutT branches sampled at 40,000 generations (Fig. 1A)."[13,14]	
glmUS prc	Encodes glucosimine	1 of 12[1]		Results are unpublished by M. Stanek, as referenced in Barrick, 09.[1] "In either case, it seems likely that the insertion reduces binding of the NagC activator to BoxG1, thereby reducing glmUS expression from the P1 promoter during growth on glucose." Identification and dynamics of a beneficial mutation in a long-term evolution experiment with Escherichia coli, Stanek, BCM Evolutionary Biology, 2009.	4.9%[1]

References:

1. Barrick, *Nature,* 09.
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3. Schneider, *Genetics* , 00.
4. Cooper, V., *Journal of Bacteriology, 01.*
5. Cooper, T., *PNAS* , 03.
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8. Lenski, *PNAS* , 94.
9. Philippe, *Journal of Bacteriology* , 09.
10. de la Rosa, *PNAS* , 85.
11. Crozat, *Genetics,* 04.
12. Blount, *Nature* , 12.
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14. Rotman, *Journal of Bacteriology, 07.*
15. NCBI *E.coli* genome database for REL606.
16. Supplementary information from reference 1.

Total Genome Size Reduction of Clones Descended From Ancestral REL606 Strain by 40K Generation:

Gene or Region	BPs Deleted
insL-2…	1
nmpC...	8,224
manB **…**	23,293
nrdE...	1
gltB...	16
kup...	6,934
pflC...	1
ydiA…	1
ogrK…	22,146
gatZ…	8,045
ECB…	61
yjaH…	1
Total Deletions	68,724 [16]
Initial Genome Size	4,629,812 bp[15]
Genome Size at 40K	4,561,088 bp