

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
<i>nadR</i>	Encodes repressor of the NAD biosynthesis genes. ³	12 of 12 ^{1,2}		"The IS150 -E insertion almost certainly inactivated <i>nadR</i> ." 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
<i>pykF</i>	Core regulatory enzyme in glycolysis metabolism. "...the <i>pykF</i> gene which encodes pyruvate kinase I, one of two glycolytic isoenzymes that catalyze the conversion of phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP." ³	12 of 12 ^{1,2}	5k -> Fixed ¹	"The insertion almost certainly inactivated <i>pykF</i> ." 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 <i>E. coli</i> ... Viewed in this light, the <i>pykF</i> :IS150 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
<i>rbs</i> op.	Involved in making the sugar ribose.	12 of 12 ¹	Fixed by 2k ⁴	"Twelve populations of <i>Escherichia coli</i> B all lost D-ribose catabolic function during 2,000 generations..." ⁴ "...involved the deletion of part or all of the ribose operon (<i>rbs</i> genes)." ⁴	2.1% ⁴
<i>malT</i> 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
<i>spoT</i>	"Encodes a protein involved in synthesis and degradation of ppGpp, the molecular 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 <i>spoT</i> mutation lowers expression of the flagella-encoding <i>flg</i> 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 <i>spoT</i> , might increase the rate of transcription 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 †
<i>pbpA</i> op.†	The <i>pbpA</i> operon encodes the PBP2 (penicillin binding protein 2) and <i>RodA</i> proteins which are involved in cell wall bio-	6 of 12 ⁹	2k-> Fixed ⁹ 5k -> Fixed ¹	"Evolved <i>pbpA</i> alleles reduce the expression of PBP2." ⁹ "Both evolved mutations in the <i>pbpA</i> promoter region caused substantial reductions in the transcription of the operon." ⁹ "The evolved <i>pbpA</i> mutations also had pleiotropic effects that were detrimental under certain other conditions. In particular, both evolved <i>pbpA</i> alleles caused increased sensitivity to	

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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 initiation 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

<i>topA</i> <i>fis</i>	<i>TopA</i> and <i>fis</i> genes affect DNA topology- control supercoiling. ¹¹ <i>TopA</i> encodes for topoisomerase I which relaxes DNA. <i>Fis</i> encodes the <i>fis</i> repressor protein that controls the level of DNA supercoiling. ¹¹	10 of 12 ¹¹ F	2k-Fixed ^{1,11}	"As shown in Figure 2, both the <i>topA</i> and <i>fis</i> 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 <i>gyrAB</i> and reduces the activity of DNA gyrase, which may explain the increased level of supercoiling caused by the evolved <i>fis</i> 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 <i>fis</i> allele had somewhat less than half the ancestral level of <i>fis</i> protein, while the <i>fis</i> -deletion strain produced no detectable <i>fis</i> ." ¹¹ "The <i>topA</i> mutation decreased the activity of the enzyme, while the <i>fis</i> mutation decreased the amount of <i>fis</i> gene product produced." ⁷	13.3% ^{1,11} 2.9% ^{1,11}
<i>citT</i> 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 <i>cit</i> amplification (<i>rnk-citT</i> module) led us to propose that the Cit+ trait arose from an amplification-mediated promoter capture." ¹² "The native <i>citT</i> regulatory region showed no expression (above background) in any strain, indicating that <i>citT</i> is normally silent under oxic conditions.... These results indicate that the <i>rnk-citT</i> module can support <i>citT</i> expression during aerobic metabolism." ¹² To summarize, in the Ara-3 clone, a 2,933 bp duplication inserted into the <i>rnk</i> gene subverting it under the regulatory control of a different pre-existing promoter (the <i>rnk</i> promoter) that lacked an "oxygen off" switch. This rearrangement resulted in the constitutive expression the <i>citT</i> operon (the Cit+ phenotype) which was further refined due to uncontrolled amplification. "Amplifications tend to be unstable." ¹² "There was	

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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

<i>mutT</i>	"The MutT protein is a hydrolase that purges the cellular nucleotide pool of oxidized guanine nucleotides (8-oxo-dGTP), which can mis-pair with adenine and lead to A:T -> C:G (adenine or thymine to cytosine or guanine) transversions after DNA replication." ¹³	1 of 12 ¹³	~20k - 25k ¹³	"...the <i>mutT</i> mutation increased the point-mutation rate by ~150 fold...." ¹³ "Moreover, hypermutators are more likely to produce offspring with deleterious or lethal mutations." ¹³ "...the <i>mutT</i> lineage had a greatly elevated mutation rate, the increased mutations caused by <i>mutT</i> hypermutators are A:T -> C:G transversions." ^{13,14} "The <i>mutT</i> mutant of <i>E. coli</i> is specifically elevated for the AT -> CG transversions and is one of the strongest mutators known, with a 10,000- to 50,000-fold increase over the wildtype." ¹⁴	
<i>mutY</i>	Encodes a DNA repair glycosylase that excises mis-paired bases. ¹³	1 of 12 ¹³	~25,633k ¹³	"Loss-of-function mutations in <i>mutY</i> , which encodes a DNA repair glycosylase that excises mis-paired bases from DNA helices, also lead to elevated mutation rates..." ¹³ "... <i>mutY</i> 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 <i>mutT mutY</i> double mutants compared with <i>mutT</i> single mutants is similar to the rate changes we observed in both phylogenomic analysis (Fig. 1B) and fluctuation tests (Fig. 1C). Indeed, genome resequencing showed that different <i>mutY</i> mutations occurred along the two <i>mutT</i> branches sampled at 40,000 generations (Fig. 1A)." ^{13,14}	
<i>glmUS</i> pro.	Encodes glucosimine	1 of 12 ¹		"In either case, it seems likely that the insertion reduces binding of the NagC activator to BoxG1, hereby reducing <i>glmUS</i> expression from the P1 promoter during growth on glucose." ¹⁷	4.9% ¹

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