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Leakiness of genetic markers and susceptibility to post-plating mutagenesis in *Escherichia coli*

R. JAYARAMAN
School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. When *Escherichia coli* strain AB1157 is subjected to starvation for threonine or leucine on solid media, threonine-independent or leucine-independent colonies continue to emerge for several days after plating. This process is strongly streptomycin dependent. Under identical conditions arginine-independent colonies do not arise when arginine starvation is imposed. Since the *thr1* and *leuB6* alleles of AB1157 could be classified as 'leaky' while the *argE3* allele cannot be so classified, there seems to be a correlation between leakiness of mutant genetic markers and post-plating mutagenesis which counters the effect of the mutations. Some of the threonine-independent variants acquired the ability to increase the leakiness of otherwise nonleaky markers such as *argE3* and permit development of arginine independence in a *recA*-dependent, *lexA*-independent manner. I show that these variants harbour a mutation, tentatively named *adi* (adaptation inducer), at around 72 min on the genetic map, and that the *adi* mutation increases the intrinsic leakiness of a *lacZ* (ochre) mutation, perhaps by enhanced translational error. These observations are discussed in relation to the phenomenon of 'adaptive' mutagenesis, its possible mechanism, and its specificity.

Keywords. Leaky mutants; adaptive mutations; post-plating mutations; Cairnsian response.

1. Introduction

A fundamental precept of neo-Darwinian evolutionary theory is the notion that mutation and selection are independent of each other. In other words the probability that a mutation occurs is independent of selection pressure or its anticipated utility. Selection is believed to be responsible merely for the steady-state distribution of variants in a population. Occurrence of mutations is also believed to be random with respect to time, locus and site of occurrence. The randomness of spontaneous mutations and their independence from selection pressure were proved by the classic experiments of Luria and Delbrück (1943), Lederberg and Lederberg (1952), and Cavalli-Sforza and Lederberg (1956). The frequency of mutants in a bacterial population is by and large a measure of the balance between the incidence of replication errors and their correction. The former depends on the fidelity of base selection during DNA replication, which, in *Escherichia coli*, depends on the integrity of the α-subunit of the DNA polymerase III holoenzyme, the product of the *dnaE* gene. *dnaE* mutants that exhibit mutator (Maki *et al.* 1991) or antimutator phenotypes (Schaaper and Cornacchio 1992; Fijalkowska *et al.* 1993; Fijalkowska and Schaaper 1993; Schaaper 1993) have been described. The mutator phenotype also ensues from defects in the *in situ* error correction carried out by the 3′-exonuclease activity of the ε-subunit of DNA polymerase, encoded by the *dnaQ* gene (Maruyama *et al.* 1983; Takano *et al.* 1986). Another source of hypermutability is the methyl-directed mismatch repair system (MMR; reviewed by Radman and Wagner 1986; Modrich 1987).

The overwhelming impact of the Luria–Delbrück experiment and the constant use of growing cells for studies on mutagenesis have led to an axiom that cells in stationary phase or under unfavourable selection do not mutate. However, several years ago Ryan and coworkers showed that cells not overtly synthesizing DNA could also suffer mutation (for references see Foster 1993). Unfortunately Ryan's experiments did not receive the attention they deserved and were hardly referred to in the literature. Shapiro (1984) pointed out that the Luria–Delbrück experiment involved the use of lethal selection which immediately killed the nonmutant cells, and therefore the inference that cells under selection do not mutate was not correct. The suggestion of a 'nonrandom and possibly product-oriented form of mutation' made by Cairns *et al.* (1988) in a provocative paper triggered off a heated debate which has not abated till date (Sarkar 1991; Keller 1992; Stahl 1992; Lenski and Mittler 1993; MacPhee 1993; Culotta 1994; Thaler 1994; Hall 1995; Sniegowski 1995; see also Hall 1990 for earlier references).

The main claim of Cairns *et al.* (1988) was that when Lac$^-$ *E. coli* was under pressure to utilize lactose for growth, Lac$^+$ variants continued to appear long after the ones present at the time of plating had shown up as colonies and that this was not accompanied by other neutral, unselected mutational events. There have been several reports of seemingly selection-specified mutations in *E. coli*, called by various names such as adaptive mutations, post-plating mutations, Cairnsian mutations and selection-specific mutations (for references see Jayaraman 1992). Whether such mutations are the result of specific and nonrandom mutagenic events or are selected out from several nonspecific and random ones has been debated (reviewed by Foster 1992, 1993). Whatever the underlying mechanism, *E. coli* seems to be capable of generating variants after cells are exposed to some selection pressure. The phrase 'post-plating mutagenesis' is used in this report in a temporal context, without implying any mechanism, to denote the emergence of variants *after* cells are plated on some medium. In an earlier report I (Jayaraman 1992) referred to my observation that some mutational markers that could be characterized as 'leaky' were hypersusceptible to post-plating mutagenesis. This report examines in detail the relation between leakiness of genetic markers and susceptibility to such mutagenesis.

2. Materials and methods

2.1 *Bacterial strains*

The *E. coli* strains used in this study are listed in table 1.

2.2 *Media*

Conventional media as described in Miller (1972) were used throughout. Nutritional supplements were used at 30 μg/ml.

2.3 *Scoring of mutants*

Cells were grown to saturation or near-saturation in complete minimal medium.

Table 1. List of *E. coli* strains.

Strain	Relevant genotype	Source/construction
AB1157	*hisG4* Δ(*gpt-proA*) *leuB6 thr1 argE3 rpsL31*	Lab collection
GW3722	Same as AB1157; *mutS* :: Tn*5*	G. C. Walker via J. Das
GW3724	Same as AB1157; *mutL* :: Tn*5*	G. C. Walker via J. Das
MV1138	Same as AB1157; *recA281*	T. Kogoma
AB1157/3	Same as AB1157; *adi*	This work
CSH34	Δ(*lac-pro*) / F′ *lacZU118 ProA*$^+$*B*$^+$	Lab collection
GJ627	*zhc904* :: Tn*10*	J. Gowrishankar
CV437	F$^-$ Δ(*lac-proAB*) *leuB6*	Lab collection
CV437 *zhc904* :: Tn*10*	Same as CV437; *zhc904* :: Tn*10*	CV437 × P1 / GJ627
JW375	*zhc511* :: Tn*10*	B. J. Bachmann
DV9	*zhc9* :: Tn*10*	B. J. Bachmann
DV12	*zhc12* :: Tn*10*	B. J. Bachmann
HfrC-1	*lexA3 malE* :: Tn*5*	Local construct
GW4212	Same as AB1157; *recA* :: cam	G. C. Walker

Appropriate dilutions were plated on selective media and incubated at 37°C. Colonies that appeared 48–72 h after plating were taken as preexisting mutants and those that appeared later than 72 h after plating were considered post-plating mutants.

All other relevant information on materials and methods is provided in the text wherever necessary.

3. Results

3.1 *Leakiness of mutant markers in E. coli strain AB1157*

AB1157 is auxotrophic for histidine (*hisG4*), proline (Δ*gpt–proA*), leucine (*leuB6*), threonine (*thr1*) and arginine (*argE3*). The extent of growth of AB1157 on minimal medium lacking any one of these amino acids can be taken as an index of the leakiness of the corresponding mutant allele. When approximately 10^6 colony forming units of AB1157 were spread on glucose minimal medium lacking one of these amino acids and incubated for 4–5 days, it was observed that a faint but distinct lawn of cells developed on medium lacking threonine or leucine whereas on medium lacking arginine, histidine or proline there was no observable growth. Therefore the *thr1* and *leuB6* alleles of AB1157 could be qualitatively classified as 'leaky' while the others could be called 'tight' mutations. However, the leakiness is not sufficient to allow formation of large, macroscopic colonies in the absence of the required nutrient.

3.2 *Acquisition of threonine independence in AB1157*

When $4–6 \times 10^6$ cells of AB1157 were plated on glucose minimal medium lacking threonine, Thr$^+$ revertants, if any, present at the time of plating showed up as colonies by 48–72 h after plating. On prolonged incubation a faint lawn of cells appeared (see above). This was followed by emergence of additional colonies. Table 2 presents data obtained in a typical experiment using six independent and parallel

Table 2. Development of threonine independence in AB1157.

Culture no.	Number of colonies	
	2 days	8 days
1	2	223
2	2	280
3	2	290
4	7	223
5	2	257
6	2	310

Cells from six independent and parallel cultures of AB1157 in glucose minimal medium (approximately 6×10^8 cfu/ml) were centrifuged down, washed once with saline, and resuspended in the original volume of saline; 0·1 ml of a 10^{-1} dilution of each culture was spread on glucose minimal medium lacking threonine. Colonies were scored 2 and 8 days after plating. The medium included streptomycin (150 µg/ml).

cultures of approximately the same cell density. All the cultures gave rise to threonine-independent colonies on prolonged incubation in the absence of the amino acid. The appearance of such colonies was time-dependent such that after 7–8 days of incubation the background lawn was dense, the number of colonies too many, and their sizes too small, thereby rendering accurate counting difficult. (It should be emphasized that until the late-appearing colonies are shown to be genetically *thr*$^+$ they cannot be called 'revertants'. Therefore the notation Thr$^+$ is used here only to denote phenotypic threonine independence.)

When the experiment was repeated but withholding leucine instead of threonine, a fairly dense lawn developed after 3–4 days of incubation such that it was not possible to observe Leu$^+$ colonies against the background lawn. On medium lacking arginine only the preexisting Arg$^+$ revertants showed up after 48–72 h. Neither a lawn developed nor did arginine-independent colonies arise on prolonged incubation. These observations suggest a possible relation between leakiness of mutant alleles and their susceptibility to post-plating mutagenesis.

Since the *thrI* mutation of AB1157 allows definite, albeit poor, growth in the absence of threonine, the post-plating, threonine-independent colonies could be the outcome of normal, or even elevated, incidence of random mutagenic events that occur as the cultures grow and threonine-independent cells are favourably selected. On the other hand, the poor growth under nutrient-starved conditions could be of a special kind, triggering either exclusive or preponderant occurrence of only favourable mutagenic events. To distinguish between these alternatives, the frequencies of two mutations (resistance to rifampicin and nalidixic acid), which are neither favourable nor adverse under the given experimental conditions and referred to here as neutral mutations, were scored in AB1157 grown in complete minimal medium and in threonine-less minimal medium. A practical difficulty in such

experiments is the low ($< 10^{-8}$) frequencies of occurrence of these mutations in cultures grown in minimal medium. Therefore these experiments were done using *mutS* and *mutL* derivatives of AB1157; these mutations cause high spontaneous-mutation frequencies. Table 3 shows the frequencies of Rif^r and Nal^r mutants in parallel cultures of AB1157 *mutS* :: Tn*5* and AB1157 *mutL* :: Tn*5*, grown in complete and threonine-less minimal medium. The data show that growth in the absence of threonine did not abolish occurrence of neutral mutations. On the contrary, it even enhanced the same slightly. The data of table 3 suggest that growth in the absence of a required nutrient is not different from normal growth with respect to occurrence of spontaneous mutations. This would support the first of the possibilities stated above.

However, a very interesting result was obtained when the same cells were examined for development of arginine independence. It was observed that cells grown in the absence of threonine showed a remarkable capacity to develop arginine independence (see data presented in table 4). This was not observed in the case of cells grown in complete medium. (Development of arginine independence was also observed in *mutS*$^+$ *mutL*$^+$ AB1157 grown in medium devoid of threonine and tested subsequently.) Furthermore, cells grown in the absence of threonine retained the capacity to develop arginine independence even after two cycles of growth in complete (nonselective) medium, indicating that the property is heritable. At least a proportion of such a population seems to have acquired a mutation or mutations to counter other stresses, a notion vindicated by the isolation of such a mutant (see below). Therefore growth of AB1157 in complete medium and growth in threonine-less medium are not the same, although the frequencies of neutral mutations might be so in the two cases. When a *recA*° (*recA* :: cam) derivative of *mutS* AB1157 was grown in threonine-less medium and examined for development of arginine independence, it was observed that the cells did not respond to arginine starvation although the mutator property was not affected (table 5). This important

Table 3. Frequencies of Rif^r and Nal^r mutants in *mutS* and *mutL* derivatives of AB1157.

		+ Thr medium			− Thr medium		
Strain	Culture no.	Titre/ml ($\times 10^{-8}$)	Rif^r/ml	Nal^r/ml	Titre/ml ($\times 10^{-8}$)	Rif^r/ml	Nal^r/ml
GW3722	1	8·1	700	400	13·1	8600	300
(*mutS* :: Tn*5*)	2	8·2	500	920	13·9	700	400
	3	10·2	320	180	12·6	300	300
	4	9·5	210	500	13·4	600	900
	5	8·2	900	700	12·1	1000	1100
GW3724	1	10·7	400	800	8·6	330	300
(*mutL* :: Tn*5*)	2	11·0	400	100	12·1	660	—
	3	10·2	600	600	11·1	200	700
	4	14·2	400	200	5·3	—	1000
	5	14·4	900	300	8·1	400	100

Approximately 10^5 cfu of the strains were spotted on complete and threonineless medium and grown for 7 days. The cells from each of the spots were scraped off and suspended in 1 ml saline, and cell titre was determined. The suspensions (0·1 ml) were plated on LB containing rifampicin (80 μg/ml) or nalidixic acid (25 μg/ml).

Table 4. Development of arginine independence in *mutS* and *mutL* derivatives of AB1157.

Strain	Culture no.	Titre/ml ($\times 10^{-8}$)	Arg^+/ml in cells grown in + Thr medium Day 3	+ Thr medium Day 10	− Thr medium Day 3	− Thr medium Day 10
GW3722 (*mutS* :: Tn*5*)	1	8·1	3300	3300	200	46000
	2	8·2	2300	2600	300	7200
	3	10·2	27500	27500	400	33000
	4	8·2	10200	10200	400	14000
GW3724 (*mutL* :: Tn*5*)	1	10·7	100	100	200	4500
	2	11·0	200	600	7	10000
	3	14·2	500	900	4500	17500
	4	14·4	400	400	<100	3900

One-tenth of a millilitre of 10^{-1} dilutions of the cultures (table 3) were plated on minimal medium lacking arginine. Arg^+ colonies were scored on day 3 and day 10 after plating.

Table 5. Abolition of development of arginine independence by *recA*° mutation in AB1157 *mutS*.

Strain	Culture no.	Titre/ml ($\times 10^{-8}$)	Rif^r/ml	Arg^+/ml Day 3	Day 10
GW3722′ (*mutS* :: Tn*5 recA*°) grown in + Thr medium	1	15·5	800	6400	6600
	2	15·8	600	3700	4000
	3	18·4	1000	1800	2100
	4	18·7	600	400	1000
GW3722′ (*mutS* :: Tn*5 recA*°) grown in − Thr medium	1	12·6	100	3100	6600
	2	15·6	400	5400	5400
	3	18·4	200	2300	2600
	4	13·4	700	1800	6800

Experimental conditions were as given under table 3. One-tenth of a millilitre was plated for scoring Rif^r and 0·1 ml of 10^{-1} dilutions were plated for scoring Arg^+ colonies.

observation suggests that acquisition of the capacity to develop arginine independence is *recA* dependent and is perhaps different from random mutagenesis which is *recA* independent (see Discussion).

3.3 *Influence of streptomycin on leakiness and acquisition of threonine independence*

AB1157 harbours the *rpsL31* mutation which confers resistance to streptomycin. In the course of these experiments it was noticed that the leakiness of mutant markers in AB1157 was dependent on the presence of streptomycin in the medium. For instance, the *thr1* and *leuB6* markers, which were leaky on streptomycin-containing medium, were 'tight' on medium lacking streptomycin. The data are presented in table 6. It can be seen that omission of streptomycin drastically curtails development

Table 6. Development of threonine independence in presence and absence of streptomycin.

	No. of Thr^+ colonies			
	– Streptomycin		+ Streptomycin	
Strain	2·5 days	8 days	2·5 days	8 days
AB1157	27	37	30	350
MV1138	6	8	10	1100

Experimental conditions were as under table 2. The data are averages for three independent cultures.

of threonine independence. The background lawn that comes up because of leakiness was also not very prominent. This was true of the *recA281* (operator-constitutive) derivative of AB1157 (MV1138) also. (The *recA281* derivative of AB1157 was examined in connection with another line of work not reported presently.)

3.4 *Isolation of a hyperleaky mutant of AB1157*

When AB1157 was plated on glucose minimal medium devoid of threonine and streptomycin, some, albeit very few, threonine-independent colonies did appear (see table 6). When 10 such colonies were picked up, grown in complete minimal medium, and tested for growth in threonine-free medium in the presence or in the absence of streptomycin, the following was observed. For reasons not known and not examined, two of the colonies were nonviable in the absence of threonine (± streptomycin) and were therefore discarded. The rest were threonine independent regardless of streptomycin, although they grew better in the presence of streptomycin. When these were tested for development of arginine and leucine independence, two were found to respond very well. One of these, referred to below as AB1157/3, was chosen for further experiments since its response was better than that of the other (data not shown). Table 7 shows the kinetics of development of arginine and leucine independence in five parallel cultures of AB1157/3. Preliminary experiments

Table 7. Development of arginine and leucine independence in AB1157/3.

Culture no.	Arg^+ colonies			Leu^+ colonies	
	2 days	7 days	12 days	2 days	5 days
1	1	93	176	1	61
2	0	56	131	3	44
3	3	51	101	3	73
4	3	181	314	0	50
5	2	82	207	4	44

Experimental details were as under table 2, except that 0·1 ml of 10^{-2} dilutions were plated. Arginine independence was scored on medium containing streptomycin and leucine independence on medium lacking streptomycin (see text).

showed that inclusion of streptomycin in the medium was necessary to score for arginine independence as it allowed formation of a faint lawn. However, streptomycin increased the leakiness of the *leuB6* mutation in AB1157/3 to such an extent that it was not possible to score leucine-independent colonies against a dense background lawn; therefore acquisition of leucine independence was scored on medium lacking streptomycin. It can be seen from table 7 that there is a time-dependent appearance of arginine-independent and leucine-independent colonies in AB1157/3. Under the same conditions the parent cells AB1157 did not yield such colonies. Even without streptomycin, the leakiness of *leuB6* in AB1157/3 was so high that it was not possible to score leucine-independent colonies accurately beyond 5 days. A *lexA3* derivative of AB1157/3 was obtained by transducing the *lexA3* allele along with the linked *malE* :: Tn*5* mutation, and development of arginine independence in AB1157/3 / *lexA3* was scored. The data are presented in table 8. It can be seen that the *lexA3* mutation did not abolish the response but only delayed its onset. In another experiment the *recA* allele of AB1157/3 was inactivated by transducing *recA* :: cam from GW4212 by P1 transduction; selection was for chloramphenicol-resistant transductants, all of which were *recA*° (total abolition of recombination proficiency). These *recA*° derivatives of AB1157/3 had also lost the ability to acquire arginine or leucine independence. Thus the phenomenon described here differs from the classical SOS response in that it is independent of *lexA* although totally dependent on *recA*.

3.5 *Approximate map location of the mutation in AB1157/3*

Visual observation of the onset of arginine independence in AB1157/3 showed that the appearance of a faint lawn preceded the emergence of colonies. With AB1157 neither a lawn developed nor did arginine-independent colonies emerge upon plating on arginine-free medium. This suggested that an increase in leakiness of the *argE3* allele is perhaps a prerequisite for development of arginine independence. A possible mechanism by which the intrinsic leakiness of mutations might be enhanced is decrease in fidelity of translation such that mutant codons could be (mis)translated

Table 8. Development of arginine independence in a *lexA3* derivative of AB1157/3.

	No. of Arg^+ colonies	
Days	AB1157/3	AB1157/3 / *lexA3*
3	7	0
5	78	17
7	168	40
9	208	90
11	—	258

Experimental details were as under table 2. Volume plated was 0·1 ml of a 10^{-2} dilution (approximately 10^6 cells). The data are means for two independent cultures.

Table 9. Mapping of the mutation responsible for threonine independence in AB1157/3.

Cross no.	Donor	Recipient	Per cent loss[a] or gain[b] of Streptomycin resistance	Per cent loss[a] or gain[b] of Threonine independence
1	CV437 *zhc904* :: Tn*10*	AB1157/3	26 (36/137)	48 (68/137)
2	JW375 (*zhc511* :: Tn*10*)	AB1157/3	43 (44/103)	79 (81/103)
3	DV9 (*zhc9* :: Tn*10*)	AB1157/3	< 1 (0/110)	13 (14/110)
4	DV12 (*zhc12* :: Tn*10*)	AB1157/3	5 (5/100)	16 (16/100)
5	AB1157/3 *zhc904* :: Tn*10*	AB1157	—	25 (15/60)
6	AB1157/3 *zhc511* :: Tn*10*	AB1157	—	76 (63/83)

P1 transductions were done according to Miller (1972). Donors 5 and 6 are Tet^r Str^r Thr$^+$ transductants from crosses 1 and 2 respectively.
[a]Crosses 1–4; [b]crosses 5–6.

as wild-type codons more frequently than usual, leading to suboptimal restoration of function. Evidently such a property could be expected to be associated with ribosomes. To examine whether the enhanced leakiness of the mutant markers in AB1157/3 is a ribosomal property, four Tn*10* insertions near the *rps* gene cluster (72 min) were transduced into AB1157/3 and tetracycline resistance was selected for. The Tet^r transductants were screened for retention or loss of streptomycin resistance as well as threonine independence. It can be seen from the data presented in table 9 that threonine independence of AB1157/3 could be lost by the introduction of DNA spanning the 72-min region of the chromosome. This shows that the property is associated with a mutation in the 72-min region. This mutation is presently designated as *adi* (adaptation inducer) in the hope that the phenomenon described presently might eventually be shown to be an 'adaptive' response to starvation stress. The *adi* mutation is closely linked to the *rpsL* locus, mutations in which confer resistance to streptomycin. The data presented in table 9 suggest the following order of markers in the region: *rpsL–adi–zhc511* :: Tn*10–zhc904* :: Tn*10–zhc12* :: Tn*10–zhc9* :: Tn*10*. By converting the contransduction frequencies to distances using the formula of Wu (1966), taking L = 2·3 min, the distance between *adi* and *rpsL31* can be calculated to be approximately 0·36 min.

The transductants that lost threonine independence also lost the capacity to develop arginine independence (data not shown). This shows that the *adi* mutation is responsible for both. If the *adi* mutation leads to threonine independence, perhaps by increasing the intrinsic leakiness of the *thrI* allele, introduction of the same into AB1157 should render transductants Thr$^+$. The data presented in table 9 (crosses 5 and 6) show that this is indeed the case. (The reasons for the quantitative discrepancy between crosses 1 and 5 are not clear.)

3.6 *The adi mutation promotes codon misreading*

To see whether the *adi* mutation increases the leakiness of mutant alleles by enhanced codon misreading, the following experiment was done. The strain CSH34

harbours an ochre (UAA) mutation (*U118*) in the *lacZ* gene on an F′ plasmid. Since CSH34 displays a clear Lac⁻ phenotype it does not possess ochre suppressor(s). Therefore the only way by which active β-galactosidase could be synthesized in CSH34 is misreading of UAA as a sense codon. In a transduction experiment P1 propagated on AB1157/3 *zhc511* :: Tn*10* (donor 6 of table 9) was used to transduce DNA into CSH34 and tetracycline-resistant transductants were selected on glycerol minimal medium supplemented with IPTG (10^{-4} M), X-gal (30 μg/ml) and tetracycline (12 μg/ml). It was found in two independent experiments that approximately 50% (32/57 and 39/80) of the Tet^r colonies turned blue over a period of 7 days, although all were white initially. This shows that the transductants receiving the *adi* mutation gradually accumulate β-galactosidase over a period of time owing to misreading of UAA as a sense codon. Such accumulation either does not occur in normal *adi*$^+$ cells or is not sufficient to impart blue colour to colonies.

3.7 *Frequency of adi mutants among threonine-independent variants*

When AB1157 is subjected to threonine starvation, it could acquire threonine independence either by true reversion, or by second-site reversion, or by the *adi* mutation. Therefore the threonine-independent colonies could be expected to fall into at least three categories. The *adi* mutants could be distinguished from the other two by their ability to develop arginine independence. True *thr*$^+$ revertants could be expected to grow better and faster than second-site revertants in the absence of threonine. In the course of this study it was observed that approximately 10% of the threonine-independent variants developed arginine independence on subsequent screening, approximately 30% appeared to be true revertants, while the rest were probably second-site revertants. The latter two categories have not been characterized further.

4. Discussion

The accumulation of mutations in bacterial and yeast cells under selection is well documented. The adherents of the adaptive mutation theory contend that only advantageous mutations accumulate in populations under selection (see Foster 1993). Even the critics of the theory do not refute the data but have only offered alternative, neo-Darwinian explanations (Lenski and Mittler 1993). Although no generalized molecular explanation, applicable to all instances of mutagenesis under selection, is yet available, in at least one case (reversion of *lacZ* frameshift mutations under lactose selection) molecular proof has been obtained for a mechanism that is different from the wide spectrum of mutational events seen in growing cells (Foster and Trimarchi 1994; Rosenberg *et al.* 1994). The time-dependent appearance of threonine-independent variants when *E. coli* AB1157 is grown on media devoid of threonine could be viewed as a response (adaptation?) of the cells to threonine starvation. That such variants do not exist before the imposition of selection is shown by non-appearance of colonies if streptomycin is omitted from the selection media. However, it could be argued that they do exist but need streptomycin for growth and therefore do not show up in its absence. This does not seem to be the case since none of the threonine-independent colonies, irrespective of time of

appearance, was dependent on streptomycin for growth. It could also be argued that a nonselectively grown population has many slow-growing, threonine-independent variants that show up late on selective media. The continuous emergence of threonine-independent colonies for several days after plating argues against this notion because, if it were true, one has to invoke a spectrum of growth rates for such variants. Therefore, in all probability, these variants seem to arise only after the onset of threonine starvation. The leaky phenotype of the *thrI* and *leuB6* mutations of AB1157 permits very slow growth in the absence of threonine or leucine. Under such conditions mutations that restore normal or near-normal growth could occur. Such mutations could be true reversions or other mutations, intragenic or extragenic, that increase the intrinsic leakiness to very high levels. While the intrinsic leakiness of the *thrI* allele of AB1157 is not sufficient for formation of large, visible colonies in the absence of threonine, the post-plating Thr^+ derivatives do form visible colonies. Some of the threonine-independent colonies do not develop independence from other auxotrophic requirements such as arginine, but some others do. The latter class has been shown in this report to harbour a lesion, tentatively named *adi* (adaption inducer) mutation, at around 72 min on the chromosome. The *adi* mutation has been shown to enhance intrinsic leakiness of mutant markers by decreasing fidelity of translation. Such decrease in translational fidelity would give rise to small amounts of normal protein by mistranslation of mutant codons. If sufficient amounts of normal protein are synthesized, it would allow suboptimal growth under selection, thereby setting the stage for occurrence of mutations that might lead to normal or near-normal growth. It has been observed in our laboratory (unpublished data) that the effect of the *adi* mutation on leakiness of mutant markers is greater under suboptimal than normal (optimal) conditions of growth. It is not difficult to visualize the benefit of such differential effect of the *adi* mutation to the organism. A low level of codon misreading during normal growth would prevent excessive accumulation of wrong proteins. Under suboptimal growth conditions, however, a 'wrong' protein might well be the one the cell needs to tide over a crisis. Once normal growth is restored the level of mistranslation could be reduced. The underlying mechanisms are not yet understood.

Earlier reports have shown that *recA* function is required for mutagenesis under selection but not for normal, random mutagenesis (Cairns and Foster 1991; Harris *et al.* 1994). It has been shown in this report that random mutagenesis during threonine starvation is not affected by loss of *recA* function whereas acquisition of the capacity to develop arginine independence is (see table 6). This shows that the two processes are mechanistically different. Therefore it is tempting to call, albeit tentatively, the post-plating mutagenesis under nutrient starvation an 'adaptive' response.

It should be noted that only a small fraction (10–20%) of the threonine-independent colonies were *adi* mutants. The majority were threonine-independent but adi^+ since they did not respond to arginine starvation. Some of them (adi^+) grew well in the absence of threonine and therefore could be true revertants. Others grew somewhat poorly in the absence of threonine and therefore are likely to be second-site revertants at the *thrI* locus. This has not yet been analysed in detail.

The map position of the *adi* mutation (approximately 72 min) suggests that it could define a ribosomal-protein gene. It is known from the classic work of Gorini and coworkers that mutations in *rpsD* and *rpsE* increase translational ambiguity

(for references see Yarus and Thompson 1983). Hence it is possible that the *adi* mutation is located in *rpsD* or *rpsE*. This needs to be verified.

Lenski *et al.* (1989) and Mittler and Lenski (1992) have proposed that, in nongrowing populations of bacteria under selection, mutations that allow slow growth could occur and such slow-growing clones could further mutate to faster growth (the 'growth on the plate' model). The intrinsic leakiness of the *thrI* mutation in AB1157 affords the same advantage as would a mutation to slow growth. Therefore when AB1157 is starved for threonine, it could acquire threonine independence either through increase of the intrinsic leakiness of *thrI* by the *adi* mutation or through secondary mutation(s) in *thrI* itself. Since only a small fraction (10%) of the threonine-independent colonies were *adi* mutants the predominant event during post-plating mutagenesis seems to involve mainly, if not exclusively, the gene under selection. Acquisition of threonine independence *via* the *adi* mutation during threonine starvation is very significant because it not only helps to tide over the immediate stress but could also be harnessed to meet other exigencies, such as arginine starvation, if they were to occur subsequently.

Mutations in *recA* not only abolish the development of threonine independence in AB1157 or arginine independence in AB1157/3 but also the leakiness of the mutations. It has also been observed in our laboratory that the leakiness of *thrI* is also influenced by the *rpsL* alleles (Jayaraman and Narendra Kumar, unpublished observations). Since leakiness of markers appears to be a prerequisite for post-plating mutagenesis these observations show that the process is subject to complex physiological control, which needs to be understood. It has been shown recently that a well-studied 'adaptive' mutagenic phenomenon, namely excision of Mu phage sequences to generate *ara–lacZ* fusions, is under such complex control (Foster and Cairns 1994; Maenhaut-Michel and Shapiro 1994).

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References

Cairns J. and Foster P. L. 1991 Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics* 128: 695–701

Cairns J., Overbaugh J. and Miller S. 1988 The origin of mutants. *Nature* 355: 142–145

Cavalli-Sforza L. L. and Lederberg J. 1956 Isolation of preadaptive mutants by sib selection. *Genetics* 41: 367–381

Culotta E. 1994 A boost for "adaptive" mutation. *Science* 265: 318–319

Fijalkowska I. J., Dunn R. L. and Schaaper R. M. 1993 Mutants of *Escherichia coli* with increased fidelity of DNA replication. *Genetics* 134: 1023–1030

Fijalkowska I. J. and Schaaper R. M. 1993 Antimutator mutations in the α-subunit of *Escherichia coli* DNA polymerase III: Identification of the responsible mutations and alignment with other DNA polymerases. *Genetics* 134: 1039–1044

Foster P. L. 1992 Directed mutation: Between unicorns and goats. *J. Bacteriol.* 174: 1711–1716

Foster P. L. 1993 Adaptive mutation: The uses of adversity. *Annu. Rev. Microbiol.* 47: 467–504

Foster P. L. and Cairns J. 1994 The occurrence of heritable Mu excisions in starving cells of *Escherichia coli*. *EMBO J.* 13: 5240–5244

Foster P. L. and Trimarchi J. M. 1994 Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. *Science* 265: 407–409

Hall B. G. 1990 Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* 126: 5–16

Hall B. G. 1995 Genetics of selection-induced mutations: I. *uvrA*, *uvrB*, *uvrC* and *uvrD* are selection-induced specific mutator loci. *J. Mol. Evol.* 40: 86–93

Harris R. S., Longerich S. and Rosenberg S. M. 1994 Recombination in adaptive mutation. *Science* 264: 258–260

Jayaraman R. 1992 Cairnsian mutagenesis in *Escherichia coli*: Genetic evidence for two pathways regulated by *mutS* and *mutL* genes. *J. Genet.* 71: 23–41

Keller E. F. 1992 Between language and science. The question of directed mutation in molecular genetics. *Perspect. Biol. Med.* 35: 292–306

Lederberg J. and Lederberg E. M. 1952 Replica plating and indirect selection of mutants. *J. Bacteriol.* 63: 399–406

Lenski R. E. and Mittler J. E. 1993 The directed mutation controversy and neo-Darwinism. *Science* 259: 188–194

Lenski R. E., Slatkin M. and Ayala F. J. 1989 Mutation and selection in bacterial populations. *Proc. Natl. Acad. Sci. USA* 86: 2775–2778

Luria S. E. and Delbrück M. 1943 Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28: 491–513

MacPhee D. G. 1993 Is there evidence for directed mutation in bacteria? *Mutagenesis* 8: 3–5

Maenhaut-Michel G. and Shapiro J. A. 1994 The roles of starvation and selective substrates in the emergence of *araB–lacZ* fusion clones *EMBO J.* 13: 5229–5239

Maki H., Mo J. Y. and Sekiguchi M. 1991 A strong mutator effect caused by an amino acid change in the alpha subunit of DNA polymerase III of *Escherichia coli*. *J. Biol. Chem.* 266: 5055–5061

Maruyama M., Horiuchi H., Makai H. and Sekiguchi M. 1983 A dominant (*mutD5*) and a recessive (*dnaQ49*) mutator of *Escherichia coli*. *J. Mol. Biol.* 167: 757–771

Mittler J. E. and Lenski R. E. 1992 Experimental evidence for an alternative to directed mutation in the *bgl* operon. *Nature* 356: 446–448

Modrich P. 1987 DNA mismatch correction. *Annu. Rev. Biochem.* 56: 435–466

Radman M. and Wagner R. 1986 Mismatch repair in *Escherichia coli*. *Annu. Rev. Genet.* 20: 523–538

Rosenberg S. M., Longerich S., Gee P. and Harris R. S. 1994 Adaptive mutations by deletions in small mononucleotide repeats. *Science* 265: 405–407

Sarkar S. 1991 Lamarck contre Darwin, reduction versus statistics: Conceptual issues in the controversy over directed mutagenesis in bacteria. In: *Organism and the origin of self* (ed.) A. I. Tauber (Dordrecht: Kluwer Academic) pp. 235–271

Schaaper R. M. 1993 The mutational specificity of two *Escherichia coli dnaE* antimutator alleles as determined from *lacI* mutation spectra. *Genetics* 134: 1031–1038

Schaaper R. M. and Cornacchio R. 1992 An *Escherichia coli dnaE* mutation with suppressor activity towards mutator *mutD5*. *J. Bacteriol.* 174: 1974–1982

Shapiro J. A. 1984 Observations on the formation of clones containing *araB–lacZ* cistron fusions. *Mol. Gen. Genet.* 194: 79–90

Sniegowski P. D. 1995 The origin of adaptive mutants: random or nonrandom? *J. Mol. Evol.* 40: 94–101

Stahl F. W. 1992 Unicorns revisited. *Genetics* 132: 865–867

Thaler D. S. 1994 The evolution of genetic intelligence. *Science* 264: 224–225

Tokano K., Nakabeppu Y., Makai H., Horuchi J. and Sekiguchi M. 1986 Structure and function of *dnaQ* and *mutD* mutators of *Escherichia coli*. *Mol. Gen. Genet.* 205: 9–13

Wu T. T. 1966 A model for three point analysis of random general transduction. *Genetics* 55: 405–410

Yarus M. and Thompson R. C. 1983 Precision of protein biosynthesis. In: *Gene function in prokaryotes* (eds.) J. Beckwith., J. Davies and J. A. Gallant (Cold Spring Harbor: Cold Spring Harbor Laboratory Press) pp. 23–63