Transient and Heritable Mutators in Adaptive Evolution in the Lab and in Nature

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ABSTRACT

Major advances in understanding the molecular mechanism of recombination-dependent stationary-phase mutation in *Escherichia coli* occurred this past year. These advances are reviewed here, and we also present new evidence that the mutagenic state responsible is transient. We find that most stationary-phase mutants do not possess a heritable stationary-phase mutator phenotype, although a small proportion of heritable mutators was found previously. We outline similarities between this well-studied system and several recent examples of adaptive evolution associated with heritable mutator phenotype in a similarly small proportion of survivors of selection in nature and in the lab. We suggest the following: (1) Transient mutator states may also be a predominant source of adaptive mutations in these latter systems, the heritable mutators being a minority (Rosenberg 1997); (2) heritable mutators may sometimes be a product of, rather than the cause of, hypermutation that gives rise to adaptive mutations.

ADAPTIVE mutations are those that allow organisms to succeed in the face of natural or artificial selections. These mutations can arise by any of several routes. "Adaptive mutation" has also been used to denote a particular set of mutational routes, described in bacteria and yeast, that differ from canonical growth-dependent mutations (*e.g.*, Luria and Delbrück 1943; Newcomb 1949; Lederberg and Lederberg 1952) in that the mutations occur after cells are exposed to selection, in cells apparently not dividing, and only had been found in genes whose functions were selected (Cairns *et al.* 1988; reviewed by Drake 1991; Foster 1993; Hall 1993; Rosenberg 1994, 1997). This past year, the best-studied assay system for such mutations also has been shown to produce unselected (nonadaptive) mutations (reviewed by Rosenberg 1997). Therefore, this special mode of mutation is now called stationary-phase, or stressful *l*ifestyle-*a*ssociated *m*utation (SLAM), of hypermutation. It may be a major route to formation of adaptive mutations as defined here.

Stationary-Phase Mutation pre-1997

The mere existence of a mutagenic mode that appeared to produce adaptive mutations preferentially was controversial because of the possibility that mutations might be directed, in a Lamarckian manner, specifically to the selected gene (Cairns *et al.* 1988). In one experimental system, the stationary-phase mutation was demonstrated to exist as a process distinct from normal growth-dependent mutation by virtue of using a different molecular mechanism of mutation. [See Maenhaut-Michel and Shapiro (1994) and Maenhaut-Michel *et al.* (1997) for evidence that stationary-phase transposon-mediated deletion mutations are also mechanistically different from those occurring during growth.]

Recombination: Stationary-phase reversion of a *lac* + 1 frameshift mutation carried on an F′ sex plasmid in *Escherichia coli* (Cairns and Foster 1991) requires functional proteins of the RecBCD double-strand break-repair recombination system, whereas growth-dependent reversion of the same allele does not (Harris *et al.* 1994, 1996; Foster *et al.* 1996). This implies that recombination is part of a unique mechanism by which the stationary-phase mutations form. Because RecBCD enzyme loads onto DNA only at double-strand ends, this also implicates DNA double-strand breaks (DSBs) as a molecular intermediate in the stationary-phase mutation mechanism (Harris *et al.* 1994). [See Taddei *et al.* (1995, 1997a) for another stationary-phase mutation assay system with RecA- and RecB-dependence that may operate via a similar mechanism to that of *lac* system.]

One way that recombination might promote mutation is illustrated in Figure 1. A recombinational strand-exchange intermediate could prime DNA synthesis. Polymerase errors made during this synthesis could become mutations. That recombinational strand-exchange intermediates are also intermediates in recombination-dependent mutation is implied by the requirement in mutation for proteins that process such intermediates (Foster *et al.* 1996; Harris *et al.* 1996) and by evidence that a transient accumulation of these intermediates is mutagenic (Harris *et al.* 1996).

That DNA synthesis is part of recombination-dependent mutation was implied by the DNA sequences of

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Although it is obvious that this issue of Genetics is dedicated to Jan Drake, the authors wish to dedicate this article to Jan and Pam Drake with heartfelt appreciation for their scrupulous and exhaustive service to the genetics community, and with particular appreciation for Jan's support and encouragement of our efforts in entering the field of mutation.

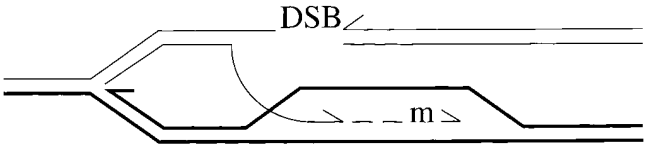

Figure 1.—Hypothesis: DNA synthesis is primed by a recombinational strand-exchange intermediate (Harris *et al.* 1994, 1996; Foster *et al.* 1996). This idea incorporates three known features of recombination-dependent mutation: the requirement for homologous genetic recombination, the involvement of DNA double-strand breaks (DSBs), and the evidence that the mutations result from DNA polymerase errors. Each line represents a single DNA strand, close parallel lines represent duplex DNA, arrowed ends represent 3′ DNA ends, dashed line represents newly synthesized DNA, and "m" indicates a DNA polymerase mistake that can become a mutation when not corrected by postsynthesis mismatch repair. As an example of what DNAs could recombine, the homologous DNAs recombining are illustrated as sister molecules derived from replication which, although drawn as uncompleted replication products, need not be. Aspects of this hypothesis that have not been tested are discussed in the text. Figure reprinted with permission from Rosenberg (1997).

the stationary-phase Lac$^+$ reversions, which are nearly all −1 deletions in small mononucleotide repeats (Foster and Trimarchi 1994; Rosenberg *et al.* 1994). These are characteristic of DNA polymerase errors and are different from growth-dependent Lac$^+$ reversions, which are heterogeneous (Foster and Trimarchi 1994; Rosenberg *et al.* 1994). The major replicative polymerase of *E. coli*, PolIII, has been implicated in the stationary-phase mutagenesis (Foster *et al.* 1995; Harris *et al.* 1997a).

The idea in Figure 1 is similar to that hypothesized to explain origin-independent, inducible stable DNA replication in *E. coli* (iSDR; Kogoma 1997), which has similar, but not identical, genetic requirements to recombination-dependent stationary-phase mutation (Asai and Kogoma 1994; Foster *et al.* 1996; Harris *et al.* 1996). For both, features that have not been tested experimentally include the following: (1) Whether there is a direct association of recombination with the DNA synthesis that leads to mutation. Alternatively, synthesis might occur in one genomic region with recombination required elsewhere, for example, for cell survival, or mutation fixation; (2) whether 3′ end invasions initiate the DNA synthesis (leading strand) as drawn. Alternatively, 5′ end invasions, evidence for which also exists (Dutreix *et al.* 1991; Rosenberg and Hastings 1991; Miesel and Roth 1996; Razavy *et al.* 1996; Roca and Cox 1997), could allow assembly of whole replication forks; and (3) whether the homologous DNAs that recombine are sister molecules as drawn. Torkelson *et al.* (1997) provide evidence that the homologies used are not regions of amplified DNA, and discuss other possible homology sources.

Are the mutations confined to sex plasmids? Although a novel mutational mechanism was obvious in recombination-dependent stationary-phase mutation, doubt was cast on the generality of this mechanism because of the possibility that the mechanism might be specific to sex plasmids. First, F′-encoded transfer functions are required for efficient Lac$^+$ reversion on the F′ (Foster and Trimarchi 1995; Galitski and Roth 1995). Second, one site on the *E. coli* chromosome (Foster and Trimarchi 1995; Radicella *et al.* 1995; R. S. Harris and S. M. Rosenberg, unpublished results), and one on the Salmonella chromosome (Galitski and Roth 1995), do not support recombination-dependent Lac$^+$ reversion. Although conjugative transfer is not required for recombination-dependent mutation (Foster *et al.* 1995; Rosenberg *et al.* 1995), the suspicion lingered that somehow bacterial sex must be responsible (*e.g.*, Galitski and Roth 1996; Benson 1997).

An alternative possibility is that the transfer (Tra) proteins promote the DSBs required for recombination-dependent mutation. Tra proteins cause single-strand nicks at the F′ origin of transfer, and these could become DSBs by any of several routes including endonuclease, or replication of the nicked template (Kuzminov 1995; Rosenberg *et al.* 1995; Rosenberg *et al.* 1996). If DSBs were limiting in the chromosome, this could account for some sites being inactive and would not exclude the possibility that DSBs formed in other ways would allow other sites to be active for recombination-dependent mutation.

Stationary-Phase Mutation in 1997

At least five important discoveries were made last year:

Not directed: Two groups provided evidence that the recombination-dependent mutation mechanism is not directed in a Lamarckian manner specifically at genes whose functions are selected. Foster (1997) measured reversion of a frameshift mutation in a defective tetracycline-resistance gene in a transposon that was hopped onto the F′ that carries *lac*. Foster found recombination-dependent accumulation of tetracycline-resistant (TetR) mutants over time after exposure to lactose as the sole carbon source, coincident with the accumulation of Lac$^+$ mutants. Independently, Torkelson *et al.* (1997) found high frequencies of unselected frameshift-reversion, loss-of-function, and temperature-sensitive mutations at sites on a cloning vector (pBR322)-style plasmid, in the bacterial chromosome, and on the F′ during recombination-dependent stationary-phase Lac$^+$ reversion. The frameshift-reversion targets used displayed a preference for −1 deletions in small mononucleotide repeats, the characteristic mutation spectrum observed previously in *lac*. These results demonstrate that the mutational process is not directed at the selected *lac* gene.

Not sex plasmid-specific: Models in which recombination-dependent stationary phase mutation was held to be specific to bacterial sex plasmids predicted that the mutagenic process would not operate on other replicons (*e.g.*, Foster and Trimarchi 1995; Galitski and Roth 1995; Radicella *et al.* 1995; Galitski and Roth

1996; Benson 1997). This contrasts with the detection by Torkelson *et al.* (1997) of high-level mutation in three replicons, including the bacterial chromosome. To compare the ability of chromosomal and F′-located sites to undergo mutation, Torkelson *et al.* (1997) screened for mutations that confer resistance to the nucleotide analog, 5-fluorocytosine (FC). These can occur by loss of function of the F′-located *codAB* locus, or by loss of function of the chromosomal *upp* gene. The FC-resistant mutations fell about equally into these two classes, as demonstrated by mapping and by a separate phenotype specific to *upp* mutants. This argues for a mechanism of chromosomal mutation similar to that on the F′. However, the recombination-dependence of the chromosomal mutations still needs to be tested.

Hypermutation in a subpopulation of stressed cells: The way that Torkelson *et al.* (1997) performed the screen for unselected mutations allowed us to observe that genome-wide hypermutation underlies stationary-phase Lac$^+$ reversion and occurs in a subpopulation of the cells exposed to selection on lactose medium. They screened for the unselected mutations by replica plating. They compared the frequency of unselected mutations among recombination-dependent Lac$^+$ mutant colonies with two important control populations: colonies from *lac*$^-$ cells that were never exposed to selection (Lac$^-$ unstressed cells) and colonies derived from Lac$^-$ cells exposed to prolonged lactose selection, rescued from the same plates that generated Lac$^+$ revertants, but which did not become Lac$^+$ (Lac$^-$ stressed cells). The Lac$^+$ revertants displayed frequencies of unselected mutations 10–100 or more times greater than in either control population, indicating that they experienced hypermutation. Moreover, starvation on lactose is not sufficient to cause hypermutation, because only some of the starved cells (the Lac$^+$ mutants) were hypermutated (Lac$^-$ stressed cells were not).

Hypermutation in the subpopulation is transient: Two possibilities for the basis of subpopulation hypermutability exist: The cells in the subpopulation could be either (1) transiently or (2) heritably hypermutable. Previously, Longerich *et al.* (1995), and more recently Torkelson *et al.* (1997), found that most recombination-dependent Lac$^+$ revertants do not possess heritable mutator phenotypes [although a small fraction of those examined by Torkelson *et al.* (1997) did, as discussed below]. However, the assays they used measured growth-dependent mutation. At least two mutator mutations that are specific to recombination-dependent mutation have been described previously: *recD* (Harris *et al.* 1994) and *recG* (Foster *et al.* 1996; Harris *et al.* 1996) null alleles. Such mutators could have been missed in tests for growth-dependent mutators.

To ask whether Lac$^+$ revertants are heritable stationary-phase mutators, we "recycled" 12 independent recombination-dependent stationary-phase Lac$^+$ revertants to Lac$^-$ and tested them in another round of stationary-phase mutation. This was done by replacing the Lac$^+$-conferring sequences with the original *lac*$^-$ frameshift mutation and then by measuring rates of stationary-phase Lac$^+$ reversion in these strains (Table 1). None of the 12 recycled stationary-phase mutants possesses a mutator phenotype for stationary-phase mutation (Table 1). This indicates that most cells that have undergone recombination-dependent stationary-phase Lac$^+$ reversion are descended from a transiently hypermutable subpopulation of all of the stressed cells. Two of the 12 are deficient in stationary-phase mutation (antimutators) (Table 1). Although the origin of the two antimutator mutations is not known, we found that the antimutator phenotype is not linked with the *lac* locus: 20 out of 20 transductants derived from each antimutator did not cotransduce the antimutator phenotype with a selectable transposon marker linked to *lac* (marker described in Table 1). It is possible that the antimutator phenotype results from unselected mutation(s) that occurred during the first round of stationary-phase mutation.

The hypermutable subpopulation: The finding of Torkelson *et al.* (1997) that unselected mutations were elevated only among Lac$^+$ revertants does not conflict with Foster's (1997) finding of unselected mutants that were Lac$^-$, because Foster's unselected mutants occurred orders of magnitude less frequently than could have been detected in the replica plating screen of Torkelson *et al.* (1997). However, Foster's data do bear on the nature of the hypermutable subpopulation discovered by Torkelson *et al.* (1997). Hall (1990) suggested that stressed cells might enter a hypermutable state that they could leave only by generating the selected (adaptive) mutation, or by death if they failed to do so. This would produce survivors that all carry an adaptive mutation, in contrast with Foster's (1997) data. Thus, current data suggest that the hypermutable state is not lethal, or at least not always lethal, in the absence of an adaptive mutation (Rosenberg 1997; Torkelson *et al.* 1997).

Hot and cold sites: The multiple chromosomal mutation targets assayed by Torkelson *et al.* (1997) varied dramatically in their mutability, showing no relationship between target size and frequency of mutation. Two fermentation regulons displayed fewer loss-of-function mutations than one single gene target. This implies the existence of hot and cold regions for the recombination-dependent stationary-phase mutation mechanism. This finding could explain the previous failure to detect recombination-dependent mutation to Lac$^+$ at the *lac* chromosomal locus (Foster and Trimarchi 1995; Radicella *et al.* 1995; R. S. Harris and S. M. Rosenberg, unpublished results) and at one locus on the Salmonella chromosome (Galitski and Roth 1995), and the previous lack of accumulation of a specific substitution mutation coincident with recombination-dependent Lac$^+$ reversion (Foster 1994). Also, stationary-phase mutation systems that operate independently of recombination

TABLE 1

Most stationary-phase Lac$^+$ revertants do not display heritable stationary-phase mutator phenotypes

Frameshift-bearing strain	Expt.	Cumulative Lac$^+$ colonies on day 5 per 10^8 viable cells[a]	Reversion relative to Tn-less parent	Reversion relative to Tn-carrying control strain	Phenotype
Tn-less parent					
FC40	1	97.9 ± 11	1		
	2	36.1 ± 8.2	1		
	3	21.5 ± 10	1		
Tn*10*-containing control strain					
SMR3739	1	158 ± 13	1.6		
	2	110 ± 14	3		
	3	62.1 ± 12	2.9		
Recycled Lac$^-$ strains					
SMR3768	1	9.1 ± 1.3		0.06	antimutator
SMR3769	1	132 ± 10		0.8	nonmutator
SMR3770	1	279 ± 35		1.8	
	3	38 ± 3		0.6	nonmutator
SMR3771	1	238 ± 31		1.5	nonmutator
SMR3772	1	14.3 ± 2.7		0.09	
	3	5.2 ± 1.5		0.08	antimutator
SMR3773	2	200 ± 64		1.8	
	3	39.3 ± 4.1		0.6	nonmutator
SMR3774	2	78.7 ± 11		0.7	nonmutator
SMR3775	2	61.5 ± 6.6		0.6	nonmutator
SMR3776	2	62.3 ± 6.3		0.6	nonmutator
SMR3777	2	43.6 ± 3.9		0.4	nonmutator
SMR3778	2	54.7 ± 7.4		0.5	nonmutator
SMR3779	2	73.4 ± 4.8		0.7	nonmutator
Nonmutator mean ±SE				0.83 ± 0.1	
Totals				10	nonmutator
				2	antimutator
				0	mutator

Twelve independent recombination-dependent stationary-phase Lac$^+$ revertants were "recycled" back to carrying their original *lac* + 1 frameshift mutation by transduction with P1 grown on *zah-281*::Tn*10 lacI33*-carrying strain SMR3739 (this work). SMR3739 was made by transduction of *zah-281*::Tn*10*, a transposon linked with *lac*, from strain CAG12080 (Singer *et al.* 1989) into the *lac* + 1 frameshift-bearing strain FC40 (Cairns and Foster 1991). By this means, each recycled Lac$^-$ strain (SMR3768-3779; this work) carries the original *lac* + 1 frameshift allele and now also carries the transposon *zah-281*::Tn*10* linked with *lac*. (The Tn was used as a selectable marker in these transductions.) These recycled Lac$^-$ strains also carry any unselected mutations that may have occurred in association with recombination-dependent hypermutation (Torkelson *et al.* 1997) and are tested here for whether or not they possess a heritable mutator phenotype for stationary-phase Lac$^+$ reversion as follows:

Recycled Lac$^-$ strains were subjected to prolonged incubation on minimal medium with lactose as the sole carbon source, and Lac$^+$ stationary-phase mutants were scored over five days' incubation as described previously (*e.g.*, Harris *et al.* 1994, 1996). For each strain in each experiment (Expt.), 8–12 independent cultures were used and the mean frequency of Lac$^+$ colonies ±1 SE is given. The presence of *zah-281*::Tn*10* on the F′ appears to cause a roughly twofold increase in stationary-phase Lac$^+$ reversion relative to the Tn-less parent. This might be caused by the transposon insertion itself or by transposon hopping or excision in stationary phase that could provide an additional source of DNA double-strand breaks, which are necessary for recombination-dependent stationary-phase mutation (Harris *et al.* 1994; reviewed by Rosenberg 1997). The recycled strains that have been through one round of recombination-dependent stationary-phase mutation, in addition to carrying *zah-281::Tn10*, do not display obvious increases in stationary-phase mutability relative to the Tn-carrying control strain. In addition, two recycled strains seem to have accrued mutations that inhibit their ability to engage in recombination-dependent Lac reversion and are designated antimutators. These two strains are not merely slow colony formers (data not shown) but appear to be genuine stationary-phase mutation-impaired strains.

[a] Values are mean ± SEM.

proteins (*e.g.*, Bridges 1993; Hall 1995; Galitski and Roth 1996) may show recombination-independence because their mutation targets reside in cold regions. [It is also possible that those with no known genetic requirements may simply assay growth-dependent mutations under circumstances in which growth is difficult to measure. However, the RecA-independent mutations of McKenzie *et al.* (1998) and Galitski and Roth (1996) do not occur during normal growth or reflect normal growth-dependent mutation rates, respectively.] Because recombination-dependent mutation requires DSBs, region-to-region variability in DSB-formation might be responsible for mutational hot and cold sites (Rosenberg *et al.* 1995; Rosenberg 1997). DSBs can be caused by arrest of replication forks (Michel *et al.* 1997), by oxidative damage (Bridges 1997), and by other enzymatic and chemical means (Rosenberg *et al.* 1996), any of which might occur heterogeneously in the bacterial chromosome. Understanding the cause of hot and cold regions will provide important insights into bacterial hypermutation.

Mismatch repair modulation: The post-replicative mismatch repair (MMR) system of *E. coli* (reviewed by Modrich and Lahue 1996) is the single largest contributor to mutation avoidance and influences multiple aspects of genetic stability, including genome rearrangement (Petit *et al.* 1991), other forms of DNA repair (*e.g.*, Lieb and Shehnaz 1995; Mellon and Champe 1996), and interspecies gene transfer (Rayssiguier *et al.* 1989; Matic *et al.* 1995, 1996; Zahrt and Maloy 1997). Homologs of the *E. coli* MMR proteins appear to play similar roles in other bacteria and in simple and complex eukaryotes (*e.g.*, Modrich 1995; Radman *et al.* 1995; Kolodner 1996). A 1997 study of recombination-dependent stationary-phase mutation in *E. coli* has produced the first evidence from any organism that MMR can be modulated and is not constitutively active (Harris *et al.* 1997b).

Transient inhibition of MMR during stationary-phase mutation was suggested by the sequences of the stationary-phase Lac^+ reversions. These are nearly all -1 deletions in small mononucleotide repeats, unlike growth-dependent Lac reversions (Foster and Trimarchi 1994; Rosenberg *et al.* 1994), but identical to growth-dependent reversions in MMR-defective cells (Longerich *et al.* 1995). Because most stationary-phase revertants are not heritably MMR-defective (Longerich *et al.* 1995; Torkelson *et al.* 1997, and Table 1 above), these results implied a transient insufficiency of MMR activity during stationary-phase mutation. Cellular levels of MutS and MutH MMR proteins were shown to decrease during the stationary phase (Feng *et al.* 1996), but did these proteins become limiting for MMR, or did they simply fall to levels appropriate for the decreased replication in the stationary phase?

Harris *et al.* (1997b) overproduced MMR proteins during recombination-dependent stationary-phase mutation, reasoning that if one were limiting, its production would restore MMR function and decrease stationary-phase mutation. MutS gave no such effect, suggesting that its stationary-phase decline is to a level appropriate for the decreased replication. MutL overproduction decreased mutation (Harris *et al.* 1997b), but the levels of measurable MutL protein remained constant during the stationary phase (Feng *et al.* 1996; Harris *et al.* 1997b). We suggest several mechanisms by which MutL might be visible on Western blots but not active for mismatch repair during stationary-phase mutation (Harris *et al.* 1997b). Whatever mechanism ultimately proves to be responsible, these results demonstrate a transient limitation of mismatch repair capacity, caused at the level of MutL protein, that is specific to stationary-phase mutation. (We show that MutL is not limiting during growth.) It remains to be tested whether the whole population or the subpopulation experiences down-modulated MMR. We have suggested previously that the subpopulation could be differentiated, not by MMR capacity, but rather by experiencing DSBs, as these are the sole loading sites for RecBCD and so represent a key control point for the ability to perform RecBCD-mediated recombination (Harris *et al.* 1994; Rosenberg *et al.* 1995; Rosenberg *et al.* 1996; Rosenberg 1997; Torkelson *et al.* 1997).

Stationary phase in bacteria is both a response to environmental conditions and a differentiated state (Seigele and Kolter 1992). After regulation of MMR in response to environmental conditions or cell differentiation would have profound consequences for evolution, development, and cancer formation. See Richard *et al.* (1997) for a similar modulation in stationary-phase human cells.

A model: A model for the mechanism of recombination-dependent stationary-phase mutation is shown in Figure 2. Some of the features of this model that have not been tested are discussed above.

Further unknowns include how a cell enters the hypermutable subpopulation. DSBs may be part of the differentiation (Rosenberg *et al.* 1994, 1996) but may not account for all of it, and moreover, how DSBs form is unknown. The DSBs may trigger an SOS response, which can be induced by starvation (Taddei *et al.* 1995) and which is required for efficient recombination-dependent hypermutation (Harris 1997), and so may be part of a signal transduction process leading to a hypermutable state. [The first links to SOS were provided by Cairns and Foster (1991). Some of these data were retracted by Foster *et al.* (1996). Data providing evidence for an SOS involvement are shown by Harris (1997).] SOS may not be the whole signal transduction pathway because there are aspects of recombinational hypermutation (*e.g.*, diminished mismatch repair) not seen in a normal SOS response. Note that standard SOS mutagenesis caused by UmuDC is not part of recombinational hypermutation (Cairns and Foster 1991). How hypermutation is regulated will have profound im-

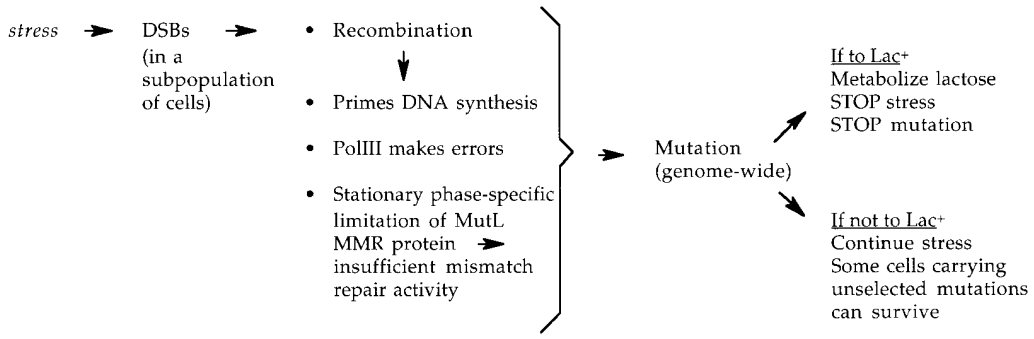

Figure 2.—A model for the mechanism of recombination-dependent stationary-phase mutation (Rosenberg 1997). Untested aspects of this model are described in the text.

plications in biology and is a subject whose surface has barely been scratched.

Transient and Heritable Mutators in Adaptive Evolution

Successful pathogenic (LeClerc *et al.* 1996) and commensal (Matic *et al.* 1997) enteric bacteria in nature, survivors of long-term laboratory culture (Sniegowski *et al.* 1997) and of selection in laboratory conditions (Mao *et al.* 1997), as well as the winners in computer-simulated selection wars (Taddei *et al.* 1997b), carry a few to several percent heritable mutator mutants in the surviving populations. Those examined so far are defective in MMR (LeClerc *et al.* 1996; Matic *et al.* 1997; Sniegowski *et al.* 1997). The heritable mutators are a minority. In these systems the mechanism(s) of accumulation of the adaptive mutations in the majority of winners has not yet been examined.

These systems seem similar to recombination-dependent hypermutation in their small percentage of heritable mutators. Torkelson *et al.* (1997) found that 6 out of 55 Lac^+ stationary-phase mutants with one or more associated unselected mutations were heritable mutator mutants. At least five of these have lost the function of an essential component of the MMR system. Four carry mutations in *mutL*, and one carries a mutation in *mutS* (Torkelson 1997). We present evidence demonstrating that the mutability experienced by most cells undergoing recombination-dependent stationary-phase mutation is transient (Table 1), and that occurs an entire novel mechanism for transient mutability in this system (reviewed above).

Several points follow from comparison of recombinational hypermutation with these other natural and laboratory systems in which mutators have been found:

1. Because of the high frequency of unselected mutations among Lac^+ stationary-phase mutants, it is possible that the few MMR-defective mutations observed are also unselected (neutral), rather than selected (beneficial) mutations. In this context it is noteworthy that antimutators are also relatively frequent (2 out of 12) among the recycled adaptive mutants in Table 1, although the frequency of antimutators among Lac^- stressed and unstressed (*i.e.*, nonhypermutating populations) cells was not measured for comparison. In the other systems showing a similarly small proportion of heritable mutators, it is also not known whether these were the cause or consequence of mutability that generated adaptive mutations.
2. In all of these systems, the ability to alter genomes radically under stressful conditions via transient, increased mutability mechanisms may be the predominant source of adaptive mutations, heritable mutators being the minority (Rosenberg 1997). That regulation, rather than heritable loss, of MMR would be more subtle and less risky has been noted (LeClerc *et al.* 1996). [See also Ninio (1991) for a specific model predicting the fraction of spontaneous mutations that should arise from transient and heritable mutators.]
3. With such normal circumstances as a commensal lifestyle being associated with apparent increased mutability, one wonders whether the laboratory may be the only environment in which selection for mutability is normally avoided.
4. Regarding the possible generality of strategies like recombination-dependent hypermutation in *E. coli*, events have been described in multicellular eukaryotes (*e.g.*, McClintock 1978) that looked similar to the radical genomic upheavals implied by the DSBs, recombination, and loss of mismatch repair. Also, association of mutations with recombination, sex, or both has been reported in bacteria (Demerec 1962, 1963), in yeast (Magni and von Borstel 1962; Esposito and Bruschi 1993; Strathern *et al.* 1995), and in filamentous fungi (Paszewski and Surzycki 1964). Finally, stationary-phase adaptive mutability has been reported in other bacteria (Kasak *et al.* 1997) and in yeast (Hall 1992; Steele and Jinks-Robertson 1992; Baranowska *et al.* 1995; Heidenreich and Wintersberger 1997), although whether these share mechanistic similarities with the system reviewed here remains to be seen.

Conclusions: Recombination-dependent stationary-phase mutation was shown this past year to be neither directed in a Lamarckian way to selected genes, nor specific to sex plasmids. Transient, genome-wide hypermutation occurs in only a subpopulation of the stressed

cells. Hot and cold sites may explain previous failures to find an unselected mutation and to find adaptive mutations in some chromosomal regions. The MMR system's activity is limited during recombination-dependent stationary-phase mutation by a lack of functional MutL, providing the first evidence that this important system for maintenance of genetic stability is not constitutive. A few heritable mutator mutants are found among stationary-phase mutants, and it is not clear whether these are selected or unselected products of the hypermutation that gives rise to the adaptive mutants. This small minority of heritable mutator mutants resembles that seen in several recent examples of adaptive evolution. This suggests that in these systems, too, most adaptive evolution may result from transient hypermutation that may occur using mechanisms similar to or overlapping with those being revealed in recombination-dependent stationary-phase mutation, a system in which the transient mutator state can be studied experimentally.

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