

Research article

Open Access

Expansion of a chromosomal repeat in *Escherichia coli*: roles of replication, repair, and recombination functions

Anthony R Poteete

Address: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA, USA

Email: Anthony R Poteete - anthony.poteete@umassmed.edu

Published: 23 February 2009

Received: 11 November 2008

BMC Molecular Biology 2009, **10**:14 doi:10.1186/1471-2199-10-14

Accepted: 23 February 2009

This article is available from: <http://www.biomedcentral.com/1471-2199/10/14>

© 2009 Poteete; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Previous studies of gene amplification in *Escherichia coli* have suggested that it occurs in two steps: duplication and expansion. Expansion is thought to result from homologous recombination between the repeated segments created by duplication. To explore the mechanism of expansion, a 7 kbp duplication in the chromosome containing a leaky mutant version of the *lac* operon was constructed, and its expansion into an amplified array was studied.

Results: Under selection for *lac* function, colonies bearing multiple copies of the mutant *lac* operon appeared at a constant rate of approximately 4 to 5 per million cells plated per day, on days two through seven after plating. Expansion was not seen in a *recA* strain; null mutations in *recBCD* and *ruvC* reduced the rate 100- and 10-fold, respectively; a *ruvC recG* double mutant reduced the rate 1000-fold. Expansion occurred at an increased rate in cells lacking *dam*, *polA*, *rnhA*, or *uvrD* functions. Null mutations of various other cellular recombination, repair, and stress response genes had little effect upon expansion. The *red* recombination genes of phage lambda could substitute for *recBCD* in mediating expansion. In the *red*-substituted cells, expansion was only partially dependent upon *recA* function.

Conclusion: These observations are consistent with the idea that the expansion step of gene amplification is closely related, mechanistically, to interchromosomal homologous recombination events. They additionally provide support for recently described models of RecA-independent Red-mediated recombination at replication forks.

Background

Expression of a chromosomal gene in *Escherichia coli* can be elevated by gene amplification. The mechanism of this amplification is thought to consist of two steps, duplication and expansion. Duplication is rare, largely *recA*-independent, and occurs between microhomologies in the chromosome as a replication accident. Expansion is frequent, *recA*-dependent, and thought to result from unequal crossing-over events between the duplicated segments [1-3].

Recent investigations of gene amplification in *E. coli* have focused on amplification of plasmid-borne genes. A phenotypically leaky F'-borne mutation, (*lacIX13-lacZ*), gives rise to Lac⁺ revertants bearing amplified arrays of 40–80 copies of the *lac* region [4]. Lac⁺ revertants of F'*lac* bearing the +1 frameshift allele (*lacI33-lacZ*), extensively employed in studies of adaptive mutation, consist mainly of one-base deletions in runs of iterated bases [5,6], but clones bearing amplified arrays appear at a lower rate as well [7,8]. Properties of *lac* amplification have generally

supported the duplication-expansion model. (i) An engineered duplication of the frameshift mutant *lac* locus amplifies at a greatly elevated frequency [9], as predicted by the idea that duplication is the rate-limiting step (and as had been seen in the case of chromosomal *ampC* [2]). (ii) Amplification is dependent upon *recBCD* and *ruvABC*, as well as *recA*, indicating an important role for homologous recombination [10].

Expansion of a pre-existing repeat has also been studied primarily on plasmids. In one study, a pBR322 derivative was constructed with two directly repeated *tetA* genes, each bearing an inactivating mutation, but arranged in such a way that a single unequal crossover would generate an array of three copies, one of which was a functioning gene. In this system, expansion was reduced only five-fold in a *recA* mutant; expansion was elevated in strains bearing mutations in *dnaQ*, *dnaE*, *dnaB*, or *dnaN* [11].

Expansion of a pre-existing duplication was compared with amplification of a single copy of F'-borne (*lacI33-lacZ*) in another study [10]. Expansion was found to be increased in a *polA* mutant, and unaffected by overexpression of *xonA*, while amplification from a single copy was inhibited by both of these conditions. It was concluded that the amplification defects caused by the *polA* mutant and by *xonA* overproduction were in duplication, not expansion.

This study was undertaken to characterize expansion of a repeated sequence in the bacterial chromosome. A duplication of chromosomal (*lacI33-lacZ*) was constructed (Fig. 1). As expected, it expands at a high rate under selection for function. The effects of mutations in various recombination, replication, DNA repair, and stress response genes on expansion of the duplication were tested. The findings support the idea that expansion occurs via homologous recombination, and suggest as well that many of the recombination events leading to expansion take place at replication forks.

Results

An *E. coli* strain bearing a chromosomal duplication of the leaky (*lacI33-lacZ*) allele, when plated on minimal medium containing lactose as the only available carbon source, gives rise to approximately 1000-fold more colonies, over the course of a week, than an otherwise isogenic strain bearing a single copy. As shown in Fig. 2, the colonies start appearing two days after plating, and accumulate at an average rate of approximately 4–5 per million viable duplication-bearing cells plated per day, two to seven days after plating. Daily colony counts vary widely between independent cultures, as well as day-to-day on the same plate. This variation is considerably greater than that observed in experiments with single copy F'-borne

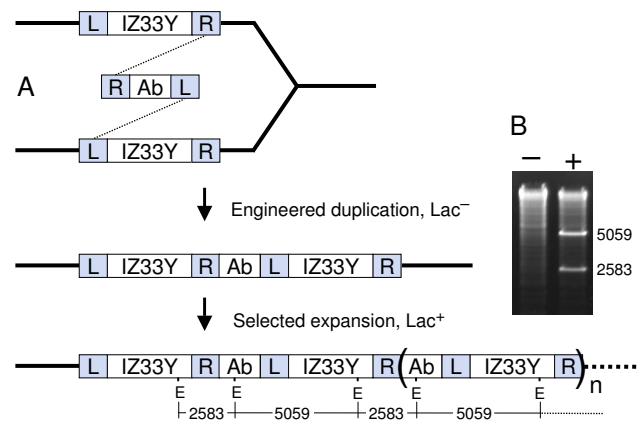


Figure 1
Expansion of a chromosomal duplication. A. Chromosomal (*lacI33-lacZ*)-*lacY* [38] was duplicated by phage λ . Red-mediated recombination with a linear DNA bearing homology-flanked antibiotic resistance marker Ab. A hypothetical mechanism by which the duplication could be generated, involving crossovers between the linear DNA and both copies of the replicating chromosomal target, is diagrammed [9]. The duplication was constructed with a tetracycline resistance element, which was later replaced with *cat*. Under selection for Lac function, the (*lacI33Y*)₂-*cat* duplication expands into multiple copies. L and R – chromosomal sequences flanking *lac*. E – EcoRI restriction sites. B. Multiple copies of the repeated sequence are seen as bands produced by EcoRI digestion of cellular DNA. Tests of two Lac⁺ revertants, one without (-), and one with (+) an expanded *lac* array, are shown as examples.

(*lacI33-lacZ*), in which the appearance of colonies after day two fits a Poisson distribution, implying that the mutations occurred after plating [12]. In contrast, the variation in colony counts seen in experiments with the chromosomal duplication strain indicate that most of the variation between cultures exists prior to plating (unpublished data). This observation is consistent with the hypothesis that each culture contains copy number variants which arise during growth, and that the probability of colony formation varies with copy number at the time of plating. Despite this variability, if 12 or more independent cultures are plated, and daily colony counts are averaged, the rate of accumulation is seen to be nearly constant, as reflected in the close fit of the data points to a straight line.

The Lac⁺ colonies appearing in these experiments can arise either by expansion or by mutation. A strain bearing even a single chromosomal copy of the un-frameshifted *lacI-lacZ* fusion grows well on lactose minimal medium. However, most or all of the excess Lac⁺ colonies produced by the duplication-bearing strain contain expanded arrays of

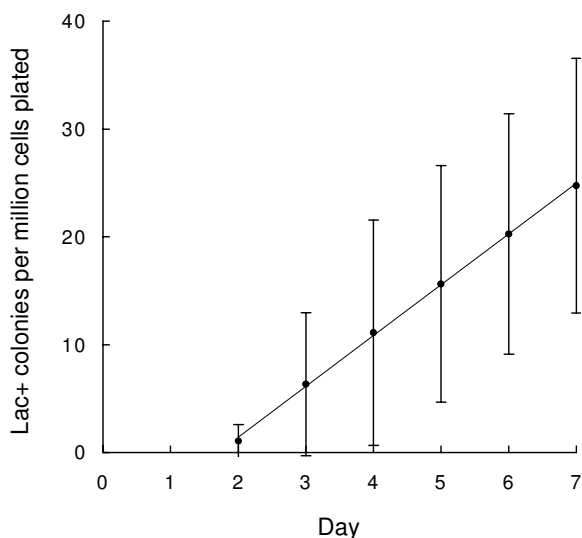


Figure 2
Kinetics of Lac⁺ colony formation. Cultures of TP1004, an MG1655 derivative bearing the *(lacZ33Y)₂-cat* duplication, were plated on lactose minimal agar. Data points represent mean daily colony counts from 19 independent cultures. Error bars represent 1 standard deviation. A least-squares linear regression curve is shown; its slope is 4.71 colonies per million viable chloramphenicol-resistant cells plated per day.

the structure diagrammed in Fig. 1. The amplified sequences in these clones are readily visualized as specific bands in restriction enzyme digests of total cellular DNA [4]. In tests of 28 Lac⁺ revertants from 22 cultures bearing the duplication, including four colonies which appeared on day two, all had expanded; none of 10 single-copy revertants contained amplified arrays (Table 1). Quantitation of total DNA, and DNA in the amplified bands, as described in the methods section, from 10 Lac⁺ clones, indicated a mean *lac* copy number of 72, with a standard

deviation of 17. Expansion of this magnitude would be expected to result in β-galactosidase production comparable to a single-copy un-frameshifted gene, as the frameshift mutation reduces β-galactosidase production 100-fold [12]. The modest variability of *lac* copy number in the Lac⁺ clones presumably reflects a sort of optimum or equilibrium, in which the benefit of more β-galactosidase is balanced by the cost of extra DNA in the chromosome (70 copies of the repeat make the chromosome roughly 10% larger). Cultures of the expanded-array variants contain unknown numbers of point-revertant *lac* genes. Under continued selection for *lac* function, it is possible that the population would eventually be taken over by revertants which, bearing a single good copy of *lac*, have the benefit of sufficient β-galactosidase without the cost of more DNA. However, it is likely that such a changeover would take many generations because the Lac⁺ revertant has, if anything, only a small selective advantage – it does not have a noticeably faster growth rate on lactose minimal medium, for example. Hastings et al. [8] tested this idea, and found that amplified *(lacI33-lacZ)* clones kept under selection for *lac* function do not form revertants readily.

The clones appearing as Lac⁺ colonies acquire their ability to grow on lactose while under selection. Colonies restreaked on lactose minimal plates form colonies visible to the unaided eye in 24 hours or less, regardless of whether they were picked on day 2 or day 7. Reversion and amplification of F'-borne *(lacI33-lacZ)* are adaptive, in that they occur only in the presence of lactose, not when the bacteria are simply starved [8,13]. The leakiness of the mutant allele is critical for adaptive mutation: residual lactose metabolism is enough to power the replication/recombination/repair processes involved, though not enough for cell division [12]. The experiment graphed in Fig. 3 shows that expansion of a chromosomal *(lacI33-lacZ)* is similarly adaptive. Cultures were plated on minimal medium containing no available carbon source. Lac-

Table 1: Expansion and survival tests

Genotype ^a	Lac ⁺ clone expansion test No. positive/no. tested	Survival on lactose minimal medium relative to wild type ^b
wild type	28/28	1
recA	0/10	0.9 ± 0.1
recBCD	9/10	0.9 ± 0.1
ruvC	10/10	1.2 ± 0.4
recG	18/18	nd
ruvC recG	6/14	0.6 ± 0.2
red+	10/10	nd
red+ recA	8/10	nd
wild type single copy	0/10	nd

a. See the legend to Figure 4 for a description of the strains tested for expansion. For the survival test, the *(lacZ33Y)₂-cat* duplication was replaced by a non-reverting *lac* deletion.

b. Means and standard errors are shown for three measurements. nd = not determined.

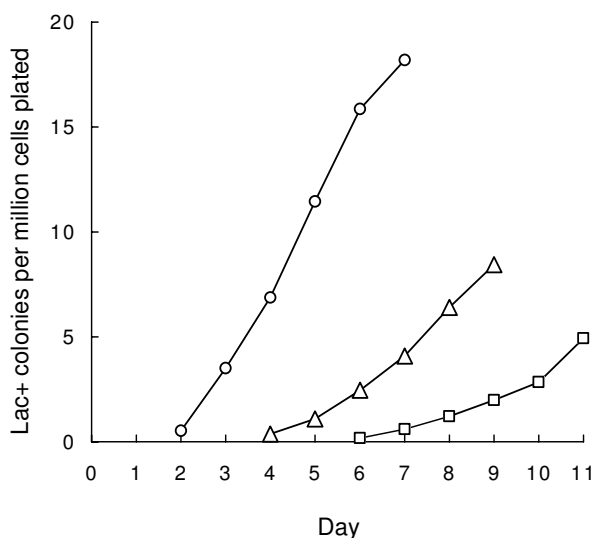


Figure 3
Adaptive nature of the expansion. Cultures of TP1004, an MG1655 derivative bearing the $(lacI33-lacZ)_2-cat$ duplication, were plated on minimal agar in which lactose was made available as the only available carbon source, either at the time of plating (circles), or after two (triangles) or four (squares) days of incubation. Data points represent mean daily colony counts from 12 independent cultures. Error bars are omitted for clarity; as in Fig. 2, the standard deviations are comparable in magnitude to the means.

tose was added after two or four days by injection under the agar slab. There was no sudden burst of colonies appearing two or three days later, as would be expected if expanded *lac* arrays had accumulated during starvation. Rather, the kinetics of appearance of Lac⁺ colonies resembled that seen in the cells initially plated on lactose, with a delay of either two or four days, and at declining rates, suggesting that the starving cells gradually lost their potential to expand.

Strains combining the duplication with mutations in DNA transaction genes were tested for Lac⁺ colony formation (Fig. 4). A null mutation in *recA* reduced the rate nearly 1000-fold, nearly down to that of a strain bearing a single copy of $(lacI33-lacZ)$ (labeled "sc" near the bottom of Fig. 4). The *recA* mutation had no significant effect in the single copy background. The strong *recA* dependence of expansion in this experiment contrasts with the weak *recA* dependence seen in a previous study of expansion of a plasmid-borne duplication [11], but is not surprising, for two reasons: (i) The duplicated segment in this study was much larger, and *recA* dependence tends to increase with increasing homology lengths [14,15]. (ii) The assay employed in the previous study required only a single recombination event, whereas becoming strongly Lac⁺ by

expansion of a chromosomal $(lacI33-lacZ)$ duplication probably involves more than one recombination event.

Null mutations in *recBCD* and *ruvC* reduced Lac⁺ colony formation 100-fold and 10-fold, respectively. Other mutations eliminating single recombination functions, *recF*, *recG*, *recN*, *recQ*, *recR*, and *ruvAB*, had little overall effect. A *ruvC recG* double mutant was also tested. Like the *recA* mutation, and as in other homologous recombination events [16], it generated Lac⁺ colonies approximately 1000-fold less efficiently than wild type.

A disruption of the *E. coli yfgL* gene was reported to confer a strong recombination/repair deficiency phenotype [17]. As shown in Fig. 4, however, a *yfgL* null mutation constructed for this study has little or no effect on expansion. It also confers no UV-sensitivity or transductional recombination phenotype, in either an MG1655 or an AB1157 strain background (not shown); others have found no recombination/repair phenotype associated with a *yfgL* null as well [18].

To test the hypothesis that the deficiencies of *recA*, *recBCD*, *ruvC*, and *ruvC recG* mutants in Lac⁺ colony formation are due to their inability to expand the duplication, two alternative explanations were considered and ruled out. (i) Lac⁺ revertants of these mutants could grow much more slowly than Lac⁺ revertants of wild type. Lac⁺ colonies were restreaked on minimal lactose plates on the days they arose. In the cases of wild type, *recA*, *recBCD*, and *ruvC*, all of the Lac⁺ clones formed colonies visible to the unaided eye by 24 hours after restreaking, independent of the day on which they arose. In the case of the *ruvC recG* double mutant, none of the 8 tested Lac⁺ revertants formed visible colonies by 24 hours, but all did so by 48 hours. However, counting the *ruvC recG* colonies after 8 days instead of 7 only increased the median from 0.029 to 0.056 colonies per million viable cells plated (data not shown). Thus, slow growth of revertants can account for only a small part of the deficiencies of the recombination mutants in forming Lac⁺ colonies. (ii) The mutants could be proficient at expansion, but deficient at survival on the selection plates. Survival of the mutants on lactose minimal medium was tested as described in the methods section. The results (Table 1) indicate that none of the mutants has a substantial survival defect relative to wild type.

The Lac⁺ revertants of the deficient mutants consist of varying populations of expanded and mutated clones (Table 1). In the case of *recA*, none of 10 tested clones had an expanded *lac* array. The frequency of expanded arrays among *recBCD* revertants was 9 out of 10; *ruvC* was 10 out of 10, *recG* was 18 out of 18, and *ruvC recG* was 6 out of 14.

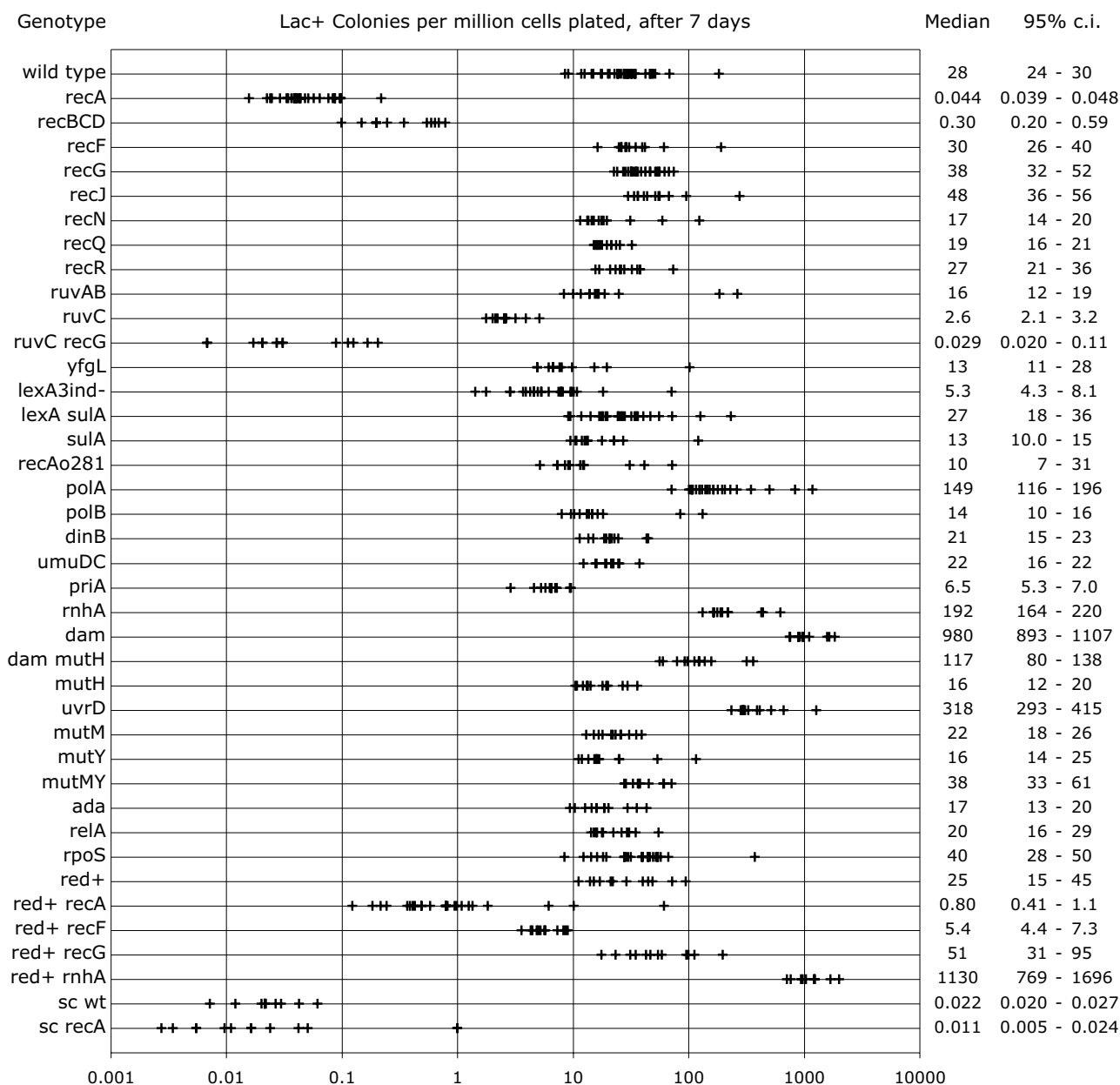


Figure 4
Roles of replication, repair, and recombination functions in expansion. Multiple independent cultures of the indicated genotype were plated on lactose minimal medium. Strains are all MG1655 derivatives. All except the ones labeled "sc" (for single copy) bear the the $(lacZ33Y)_2-cat$ duplication. Strains labeled "red+" bear the phage λ red recombination genes, which replace the *recC-ptr-recB-recD* gene cluster in the *E. coli* chromosome. Except for the red substitution, the *lexA* alleles, and the *recAo281* operator mutation, all the alleles are nulls made by substituting an antibiotic resistance element for the coding sequence of the gene.

The Lac+ reversion phenotype of a *recG* null mutant is more complex than the data in Fig. 4 suggest. The mutant strain's rate of colony formation tends to increase sharply late in the experiment, with the new colonies tending to appear as satellites of older colonies (not shown). Factors

influencing the timing and extent of this satellite-based population explosion include plating density, but are otherwise unknown. The *recG* mutant data shown in Fig. 4 are from selected experiments, in which the plating density was low, and satellitism was not as strongly evident as in

other experiments. Satellitism of this sort suggests that something produced by the older colonies on the plate stimulates recombination, perhaps via a genotoxic effect. It is consistent with the finding that overexpression of *recG* protects *E. coli* against weak organic acids [19].

The effects on expansion of varying RecA activity were tested by plating mutants affecting *recA* expression. The uninducible *lexA3* mutation has been reported to reduce the frequency of a number of different homologous recombination events [20]; it also reduced Lac⁺ colony formation approximately five-fold. The SOS-constitutive *lexA71::Tn5* mutation (in a *sulA* null background, to suppress its lethality) had no significant effect; neither did the *recAo281* operator constitutive allele.

A number of replication genes were tested for roles in expansion. A null mutation in *polA* increased the rate of expansion 5-fold; null mutations in the other non-essential DNA polymerase-encoding genes *polB*, *dinB*, and *umuDC*, had little or no effect. Loss of the replication restart function *priA* caused a small decrease in expansion efficiency, while loss of *rnhA* caused a nearly 7-fold increase.

A strain lacking *dam* function exhibited a 35-fold elevated rate of Lac⁺ colony formation. Apparently, part of this elevated rate is due to the double-strand breaks which occur as the result of mis-directed mismatch repair in *dam* mutants [21,22]. As shown in Fig. 4, a *dam mutH* double mutant exhibited an intermediate rate between those of wild type and the *dam* single mutant. The *mutH* null allele by itself had little or no effect.

Other DNA repair functions were tested for effects on expansion as well. A *uvrD* null mutant formed Lac⁺ colonies at a 10-fold elevated rate, while *mutM*, *mutY*, a *mutM mutY* double, and an *ada* null mutation had no significant effects.

The question of whether expansion of a chromosomal repeat occurs as part of a stress response, like amplification starting from a single episomal copy [23], was explored by testing null mutations in *rpoS* and *relA*. As shown in Fig. 4, these mutations had little or no effect on Lac⁺ colony formation.

Expansion mediated by the Red recombination system of phage λ was studied in a series of strains in which the phage *red* genes replace the *recC-ptr-recB-recD* gene cluster in the *E. coli* chromosome (designated "red+" in Fig. 4). Replacing RecBCD with Red has no effect on the rate of Lac⁺ colony formation, but it changes the extent to which expansion is dependent upon other recombination functions. In the *red*-substituted background, a *recA* null mutation reduces Lac⁺ colony formation only 35-fold. Among

the *recA* revertants, 8 of 10 that were tested contained expanded *lac* arrays (Table 1), showing that Red, unlike RecBCD, can promote expansion in the absence of RecA. Red-mediated expansion is reduced by a *recF* null mutation, and elevated slightly by a *recG* null; these mutant effects are seen in Red-mediated gene replacement events as well [24]. The *rnhA* null mutation has a stronger effect in the *red*-substituted background than in wild type, elevating the rate of expansion 45-fold.

Discussion and conclusion

The genetic requirements of homologous recombination in *E. coli* vary with the particular event examined, but some features of chromosomal events are nearly general: dependence on *recA* and *recBCD*, and mild or no dependence upon a variety of other recombination functions whose roles are revealed mainly in the absence of *recBCD* function [14]. Expansion by duplicated chromosomal (*lacI33-lacZ*) fits this general pattern. Similarly, mutations with known hyper-rec phenotypes – *polA*, *dam*, *uvrD*, and *rnhA* [20,25,26] – also cause an elevated rate of expansion. These observations support the idea that expansion is best understood as a homologous recombination event or series of events.

The Red recombination system of phage λ promotes RecA-independent recombination between chromosomes if at least one of the chromosomes is replicating, and RecA-dependent recombination between non-replicating chromosomes [27-30]. The RecA-independent expansion seen in *red*-substituted bacteria suggests that at least some of the Red-mediated recombination events involved in expansion take place at replication forks [30,31].

The involvement of replication in recombination events leading to expansion is additionally suggested by the increased rates of expansion of the *rnhA* and *dam* mutants. Replication in both these mutants escapes cell cycle regulation. In an *rnhA* mutant, unsynchronized DNA replication initiates at multiple sites in the chromosome [32]. In a *dam* mutant, initiation is confined to *oriC* but is not regulated [33]. Elevated recombination frequencies in both of these mutants may be due, at least in part, to an increased occurrence of double strand breaks. Both exhibit greatly reduced viability in the absence of RecBCD function, possibly because RecBCD is needed to repair the excess double strand breaks [34,35]. Eliminating the mismatch repair endonuclease MutH in a *dam* mutant prevents the double strand breaks which result from mis-directed mismatch repair, but does not bring expansion down to the wild type level; unregulated replication itself is a possible cause of the residual excess expansion in the *dam mutH* double mutant. The mechanism by which improperly regulated replication forks provoke more recombination events than normally regulated replication forks is unknown, but there are a number of possible

Table 2: Strains

Strain	Relevant Genotype	Reference, source, or construction
FC691	lacI _{Z33} ^a	[38]
GM3819	damΔkan	M. Marinus
GM8291	polAΔfrt-kan/F' polA ⁺ camR	M. Marinus; polA allele [39]
MG1655	wild type	
MV1132	srl300::Tn10 recAo281	M. Volkert
MV1154	lexA3	M. Volkert
MV2104	lexA71::Tn5	M. Volkert
TP538	recGΔtet	[40]
TP539	recGΔkan	[40]
TP540	ruvABΔtet	[40]
TP547	red-cat ^b	derivative of KM32 [41]
TP577	recFΔtet	[40]
TP605	sulAΔtet	[40]
TP643	recQΔtet	[24]
TP645	recRΔtet	[24]
TP662	sulAΔkan	substitution of Tn903 aph for Tn10 tetRA in sulAΔtet [40]
TP664	recNΔtet	[42]
TP730	red-cat lacI _{Z33}	FC691 × P1•TP547
TP732	red-pae-cl ^b lacI _{Z33}	TP730 × pTP822 linear [41]
TP796	recAΔtet	[43]
TP797	ruvCΔtet	[43]
TP798	red-cat	[36]
TP832	red-amp ^b	[36]
TP838	recBCDΔtet	[43]
TP872	red-amp lacΔcat	TP832 × cat15,16 pcr of Tn9 ^c
TP889	lacΔcat	MG1655 × P1•TP872
TP922	red-pae-cl ^b (lacI _{Z33Y}) ₂ -tet-oriR6K ^ã ^d	TP732 × pTP1061 linear ^e
TP929	(lacI _{Z33Y}) ₂ -tet-oriR6Kgamma	TP889 × P1•TP922
TP942	red-pae-cl (lacI _{Z33Y}) ₂ -cat	TP922 × TocI,2 pcr of Tn9
TP997	galK::aacC1067	[44]
TPI000	dinBΔtet	TP798 × din7,8 pcr of Tn10
TPI003	lacΔspc	TP889 was transduced with a P1 lysate of an unnamed intermediate strain, TP798 × LAT2,3 pcr of Tn21 aadA
TPI004	(lacI _{Z33Y}) ₂ -cat	TP929 × P1•TP942
TPI005	(lacI _{Z33Y}) ₂ -cat dinBΔtet	TPI004 × P1•TPI000
TPI006	(lacI _{Z33Y}) ₂ -cat recAΔtet	TPI004 × P1•TP796
TPI007	(lacI _{Z33Y}) ₂ -cat recFΔtet	TPI004 × P1•TP577
TPI008	(lacI _{Z33Y}) ₂ -cat recGΔtet	TPI004 × P1•TP538
TPI009	(lacI _{Z33Y}) ₂ -cat recNΔtet	TPI004 × P1•TP664
TPI011	(lacI _{Z33Y}) ₂ -cat recQΔtet	TPI004 × P1•TP643
TPI012	(lacI _{Z33Y}) ₂ -cat recBCDΔtet	TPI004 × P1•TP838
TPI014	(lacI _{Z33Y}) ₂ -cat ruvABΔtet	TPI004 × P1•TP540
TPI015	(lacI _{Z33Y}) ₂ -cat ruvCΔtet	TPI004 × P1•TP797
TPI020	(lacI _{Z33Y}) ₂ -cat recRΔtet	TPI004 × P1•TP645
TPI022	lacI _{Z33}	Spontaneous chloramphenicol-sensitive TPI004 derivative
TPI031	red-cat recJΔtet	TP798 × recJ1,2 pcr of Tn10
TPI032	red-cat rhnAΔtet	TP798 × rhnA1,2 pcr of Tn10
TPI033	red-cat polBΔtet	TP798 × polB1,2 pcr of Tn10
TPI034	(lacI _{Z33Y}) ₂ -cat recJΔtet	TPI004 × P1•TPI031
TPI035	(lacI _{Z33Y}) ₂ -cat rhnAΔtet	TPI004 × P1•TPI032
TPI036	(lacI _{Z33Y}) ₂ -cat polBΔtet	TPI004 × P1•TPI033
TPI038	red-amp (lacI _{Z33Y}) ₂ -cat	TP832 × P1•TP942
TPI042	(lacI _{Z33Y}) ₂ -cat damΔkan	TPI004 × P1•GM3819
TPI043	(lacI _{Z33Y}) ₂ -cat sulAΔtet	TPI004 × P1•TP605
TPI047	(lacI _{Z33Y}) ₂ -cat uvrDΔtet	TPI004/pKM208 × uvrD1,2 pcr of Tn10
TPI048	(lacI _{Z33Y}) ₂ -cat sulAΔtet lexA71::Tn5	TPI043 × P1•MV2104
TPI049	(lacI _{Z33Y}) ₂ -cat umuDCΔtet	TPI004/pKM208 [45] × umDC1,2 pcr of Tn10
TPI050	(lacI _{Z33Y}) ₂ -cat malFΔspc	TPI004/pKM208 × malF1,2 pcr of TP997
TPI051	(lacI _{Z33Y}) ₂ -cat rpoSΔtet	TPI004/pKM208 × rpoS1,2 pcr of Tn10
TPI053	(lacI _{Z33Y}) ₂ -cat adaΔkan	TPI004/pKM208 × ADA1,2 pcr of Tn903 aph

Table 2: Strains (Continued)

TPI054	(<i>lacI</i> Z33Y) ₂ -cat <i>priA</i> Δkan	TPI004/pKM208 × <i>priA</i> 1,2 pcr of Tn903 aph
TPI055	(<i>lacI</i> Z33Y) ₂ -cat <i>relA</i> Δkan	TPI004/pKM208 × <i>relA</i> 1,2 pcr of Tn903 aph
TPI056	(<i>lacI</i> Z33Y) ₂ -cat <i>sulA</i> Δtet <i>priA</i> Δkan	TPI043 × PI•1054
TPI057	red-amp (<i>lacI</i> Z33Y) ₂ -cat <i>recA</i> Δtet	TPI038 × PI•796
TPI058	red-amp (<i>lacI</i> Z33Y) ₂ -cat <i>recF</i> Δtet	TPI038 × PI•577
TPI059	red-amp (<i>lacI</i> Z33Y) ₂ -cat <i>recG</i> Δtet	TPI038 × PI•538
TPI061	red-amp (<i>lacI</i> Z33Y) ₂ -cat <i>rnhA</i> Δtet	TPI038 × PI•1035
TPI062	<i>lacI</i> Z33 <i>recA</i> Δtet	TPI022 × PI•796
TPI063	(<i>lacI</i> Z33Y) ₂ -cat <i>recA</i> Δkan	TPI004/pKM208 × <i>recA</i> 3,4 pcr of Tn903 aph
TPI064	(<i>lacI</i> Z33Y) ₂ -cat <i>mutH</i> Δtet	TPI004/pKM208 × <i>mutH</i> 1,2 pcr of 1016
TPI065	(<i>lacI</i> Z33Y) ₂ -cat <i>mutM</i> Δtet	TPI004/pKM208 × <i>mutM</i> 1,2 pcr of Tn10
TPI066	(<i>lacI</i> Z33Y) ₂ -cat <i>mutY</i> Δkan	TPI004/pKM208 × <i>mutY</i> 1,2 pcr of Tn903 aph
TPI069	(<i>lacI</i> Z33Y) ₂ -cat <i>mutM</i> Δtet <i>mutY</i> Δkan	TPI065 × PI•TPI066
TPI080	<i>lac</i> Δcat <i>recA</i> Δtet	TP889 × PI•TP796
TPI081	<i>lac</i> Δcat <i>recBCD</i> Δtet	TP889 × PI•TP838
TPI082	<i>lac</i> Δcat <i>ruvC</i> Δtet	TP889 × PI•TP797
TPI086	(<i>lacI</i> Z33Y) ₂ -cat <i>polA</i> Δfrt-kan	TPI004 × PI•JW3835
TPI089	(<i>lacI</i> Z33Y) ₂ -cat <i>yfgL</i> Δkan	TPI004/pKM208 × <i>yfgL</i> 1,2 pcr of Tn903 aph
TPI090	(<i>lacI</i> Z33Y) ₂ -cat <i>lexA</i> 3	TPI050 × PI•MV1154, selection for Mal ⁺ , screen for UV-sensitivity
TPI091	(<i>lacI</i> Z33Y) ₂ -cat <i>dam</i> Δkan <i>mutH</i> Δtet	TPI064 × PI•GM3819
TPI095	(<i>lacI</i> Z33Y) ₂ -cat <i>srl300::Tn10</i> <i>recAo281</i>	TPI063 × PI•MV1132
TPI098	(<i>lacI</i> Z33Y) ₂ -cat <i>ruvC</i> Δtet <i>recG</i> Δkan	TPI015 × PI•TP539
TPI099	(<i>lacI</i> Z33Y) ₂ -cat <i>recAo281</i>	TPI095 × PI•TPI063, selection for <i>Srl</i> ⁺ , screen for kanamycin sensitivity and the <i>Sph</i> site created by the <i>recAo281</i> mutation [46]

Notes

- The abbreviation *lacI*Z33 is used to denote the triple mutant (*lacI*33-*lacZ*) bearing the *lacI*^Q promoter-up mutation in the *lacI* promoter, a 212-bp deletion fusing *lacI* to *lacZ*, and a +1 frameshift mutation in the *lacI* sequences near the fusion junction [13].
- Strains designated "red" bear the phage λ *red* recombination genes, which replace the *recC-ptr-recB-recD* gene cluster in the *E. coli* chromosome.
- Primers used in polymerase chain reactions to generate antibiotic cassettes for gene replacements are described in Table 3.
- Strains designated "(*lacI*Z33Y)₂-" bear a duplication of *lacI*Z33 and *lacY*, with the genetic element listed after the hyphen (*tet-oriR6Kgamma* or *cat*) inserted between the two copies.
- Details of this construction are given in the text.

explanations. They might be more prone to breaking down or to running into each other, or just more numerous in the cell.

Methods**Duplication of (*lacI*33-*lacZ*)-*lacY***

Plasmid pTP1029 [36] is a vector containing the *tetRA* genes from Tn10 and the *pir*-dependent replication origin R6K*origamma*. pTP1060 was made by ligating a synthetic DNA made from two oligodeoxyribonucleotides, GATC-CAGGTTCTTTGAGCTCTTTGGCGGCCCGC and GATCGC GGCCGCCAAAGAGCTCAAAGAACCTG, into the *Bam*HI site of pTP1029. pTP1016 [36] contains *E. coli* sequences which normally flank the *lacI* and *lacY* genes; in the plasmid, they flank the *cat* gene. pTP1027 [36] and pTP1049 [37] contain the same flank sequences as pTP1016, with wild type *lacIZY* and (*lacI*33-*lacZ*)-*lacY*, respectively, between them. pTP1061 was made by ligating the *cat*-with-*lac*-flanks cassette of pTP1016 into the *Not*I site of pTP1060. A strain bearing a duplication of the chromosomal segment containing (*lacI*33-*lacZ*)-*lacY* was constructed by electroporating strain TP890 with *Bsr*G1- and *Sph*1-digested plasmid pTP1061, and selecting for tetracycline resistance (see Fig. 1). The *tet-origamma* insert between the duplicated *lac* copies was replaced by *cat* via

recombination with a linear DNA generated by PCR with a Tn9-containing strain as template, and primers GATCCCGCGGAATAACATCATTTGGTGACGAAATAA C TAAATGAGACGTTGATCGGCAGC and CCACGATGC GT CCGGCGTAGAGGATCTGAAGATCAGCA GTATTCAGGC GTAGCACCAGGC. The presence of duplicated segments in bacterial chromosomes was verified by the use of PCR with divergent primers, as described [36]. Other strain construction details are given in Tables 2 and 3.

Plating methods

Strains to be tested for reversion to Lac⁺ were grown to saturation in M9 0.1% glycerol minimal medium at 37°C, and plated on M9 0.1% lactose plates at 37°C. M9 minimal media, supplemented with thiamine at 5 μg/ml, were as described [7]. In most cases, viable duplication-positive titers were determined by plating on LB agar supplemented with chloramphenicol at 10 μg/ml, which permits colony formation only by bacteria retaining the *cat* gene between the duplicated segments. Strains with poor viability in rich media (*ruvC* *recG*, *polA*, *priA*) were titered on M9 glucose plates; retention of the duplication in these cases was assessed by testing the chloramphenicol resistance of individual colonies from the titer plates. Lactose minimal plates were inoculated with 1–2 × 10⁹ cells,

Table 3: Primers

Primer	Sequence
ADA1	GATTATGAAAAAAGCCACATGCTTAACTGACGATCAACGCACGTTGTGTCTCAAATCTC
ADA2	CTCCTCATTTTCAGCTTCGCGGCGCAGCAGTTGCGCTTTACAACCAATTAACCAATTCTG
cat15	TCTGGTGGCCGGAAGGCGAAGCGGCATGCATTTACGTTGAATGAGACGTTGATCGGCACG
cat16	AGAGTACATCTCGCGTTTTTTCTCAATTCATGGTGTACAATTCAGGCGTAGCACCAGGC
din7	GCTGGATAAGCAGCAGGTGCTTTCGCAGCGAACCGCTTAACTCGACATCTTGGTTACCGT
din8	ACCAGTTGTCTTTCCATTTGCGGGTCAAGCAACGTCACATCGCGGAATAACATCATTGG
LAT2	AAGAAAGCCTGACTGGCGTTAAATTGCCAACGCTTATTATTTGCCGACTACCTTGG
LAT3	GACGGGTGTTACTCGCTCACATTTAATGTTGATGAAAGCAAACGGATGAAGGCACGAA
malF1	GTCCTGGAATGAGGAAGAACCCCATGGATGTCATTAAAAAAACGGATGAAGGCACGAA
malF2	CCCTTAATCAAACCTTCATTCGCGTGGCTTTCAGGTTCACTTTATTTGCCGACTACCTTGG
mutH1	TTTTTAATCAAGGTATCATGACATGTCCAACCTCGCCCCTCGACATCTTGGTTACCGT
mutH2	GCGATGGCTACTGGATCAGAAAATGACGGGCCAGTAGTGCCGCGGAATAACATCATTGG
mutM1	GCATCTGTTCACTCCTGGAGATGCTATGCCTGAATTACCCCTCGACATCTTGGTTACCGT
mutM2	TCCGGCGCATGAATTACTTCTGGCACTGCCGACAATAACGCGGAATAACATCATTGG
mutY1	CAACAGTGAATTTCGGTGACCATGCAAGCGTCGCAATTTTACGTTGTGTCTCAAATCTC
mutY2	CTTTATCGACTCACGCGCTAAACCGGCGCGCCAGTGCGTACAACCAATTAACCAATTCTG
polB1	GTGGCGCAGGCAGTTTTATCTTAACCCGACACTGGCGGGCTCGACATCTTGGTTACCGT
polB2	AGTGGTGAACGTTGGTAGTCCAGCGGCTCCGGGCCGTTGGCGCGGAATAACATCATTGG
priA1	GATGCTATGCCCGTTGCCACGTTGCCTTGCCCGTTCCGCACGTTGTGTCTCAAATCTC
priA2	GGAATCCGGTATTGATTGATGAGCGCCAGCGTACCGTTACAACCAATTAACCAATTCTG
recA3	TTACCCGGCATGACAGGAGTAAAAATGGCTATCGACGAACACGTTGTGTCTCAAATCTC
recA4	CCCTTGTGTATCAAACAAGACGATTAATAATCTTCGTTTACAACCAATTAACCAATTCTG
recJ1	ACAGATACAACTTCGTCGCCGTGAAGTCGATGAAACGGCACTCGACATCTTGGTTACCGT
recJ2	GCAGTGGACCGCCGCCGACCGTTGACCATCACCTTCAACGCGGAATAACATCATTGG
relA1	GGACGATGGTTGCGGTAAAGAAGTCACATATCAATAAGGCACGTTGTGTCTCAAATCTC
relA2	GGTCATGTGCGATGGTGCACCAGTTGCTGTTGGTGTGCTACAACCAATTAACCAATTCTG
rnhA1	GGCAATCCAGGACCTGGGGTTACGGCGCTATTTACGCTCTCGACATCTTGGTTACCGT
rnhA2	GCCGCGGCAGCGGAGTTTCATCACAGCGTTGTTTTCCGCGCGGAATAACATCATTGG
rpoS1	CGGGTAGGAGCCACCTTATGAGTCAGAATACGCTGAAAGTCTCGACATCTTGGTTACCGT
rpoS2	CCTTTCTGACAGATGCTTACTTACTCGCGGAACAGCGCTTCGCGGAATAACATCATTGG
Toc1	GATCCCGCGGAATAACATCATTGGTACGAAATAACTAAATGAGACGTTGATCGGCACG
Toc2	CCACGATGCGTCCGGCGTAGAGGATCTGAAGATCAGCAGTATTCAGGCGTAGCACCAGGC
umDC1	CAGATTATTATGTTGTTTATCAAGCCTGCGGATCTCCGCGCTCGACATCTTGGTTACCGT
umDC2	CCTGCCGCTATATTTATTTGACCCTCAGTAAATCAGAACTCGCGGAATAACATCATTGG
uvrD1	AACCTATTTTTACGCGCGGTGCCAATGGACGTTTCTTACCTCGACATCTTGGTTACCGT
uvrD2	CTGGCCCTGAAATGCCACCTGCAAACGGCTATGCTCACCGCGCGGAATAACATCATTGG
yfgL1	TCTGAGAGGGACCCGATGCAATTGCGTAAATTAAGTGTGACGTTGTGTCTCAAATCTC
yfgL2	GCCGTCAGCGGCAACCGTTAGTCTGGAACCGGAACTACAACCAATTAACCAATTCTG

either of the strain to be tested by itself, or, in most cases, of the strain to be tested plus a non-reverting, *lac* deletion-bearing scavenger strain [13]. The rates of appearance of Lac⁺ colonies shown in Figures 2, 3, and 4 are calculated as Lac⁺ colonies per million chloramphenicol-resistant viable cells plated (or per million viable cells, in the cases of the single copy strains).

Expansion test

Revertant colonies appearing on the M9 lactose plates were streaked on M9 lactose plates, which were incubated at 37°C until visible colonies formed. Heavy inocula constituting the bulk of the growth from the streaks were then scraped from the plate and grown to saturation in 5 ml M9 0.1% lactose minimal medium at 37°C. (Some of the revertant colonies tested, in the wild type background

only, were inoculated directly from the selection plates into liquid lactose minimal medium; but all revertants in the wild type background tested positive for expanded arrays, regardless of the variation in culture methods). DNA was extracted by the use of a procedure involving freezing and thawing, lysozyme digestion, extraction with a phenol/chloroform/isoamyl alcohol mixture, extraction with ether, and precipitation with ethanol [30]. Portions were digested with EcoR1 and RNase, and subjected to electrophoresis in an agarose gel, followed by ethidium bromide staining. For quantitation of the repeat-specific bands, standards consisting of HindIII-digested phage lambda DNA of known concentration were included in the gel, in separate lanes. Total DNA in the sample was quantitated by spotting RNase-treated samples on an agarose slab containing ethidium bromide at 1 µg/ml, along

with standards of known concentration. Band and spot intensities were measured by the use of digital photography and Kodak 1D software.

Survival test

Mutations to be tested for their effects on survival on lactose minimal medium were crossed into a strain bearing a deletion of the *lac* operon. Cultures were grown to saturation in M9 0.1% glycerol as in the Lac⁺ reversion test, and deposited on the surfaces of 0.6 ml M9 lactose agar plugs at the bottom of 12 × 75 mm plastic tubes, at approximately the same plating density (relative to volume of medium) as the bacteria in the Lac⁺ reversion test. The tubes were incubated at 37°C. Cells were suspended by vortexing in 2 ml of buffer, and titered on M9 glucose plates, on days 0 and 7. The ratio of titer on day 7 to titer on day 0 for the wild type control was 0.83 ± 0.12 (mean ± standard error from six measurements).

Abbreviations

Kbp: kilobasepair.

Acknowledgements

I thank Patricia Foster, Michael Volkert, Martin Marinus, and Susan Lovett for helpful discussions; Michael Volkert and Martin Marinus for strains; and Rosemary Proff, Dery Miller, Shilpa Nadimpalli, Bach Nguyen, Juliet Zhang, Aiden Galarza, and Matthew Lim for technical assistance. This research was supported by US National Science Foundation grant MCB-0234991, and by a grant from the University of Massachusetts Healey Endowment.

References

- Edlund T, Normark S: **Recombination between short DNA homologies causes tandem duplication.** *Nature* 1981, **292**:269-271.
- Edlund T, Grundstrom T, Bjork GR, Normark S: **Tandem duplication induced by an unusual ampA1-, ampC-transducing lambda phage: a probe to initiate gene amplification.** *Mol Gen Genetics* 1980, **180**:249-257.
- Andersson RP, Roth JR: **Tandem genetic duplication in phage and bacteria.** *Annu Rev Microbiol* 1977, **31**:473-505.
- Tlsty TD, Albertini AM, Miller JH: **Gene amplification in the lac region of E. coli.** *Cell* 1984, **37**:217-224.
- Rosenberg SM, Longrich S, Gee P, Harris RS: **Adaptive mutation by deletions in small mononucleotide repeats.** *Science* 1994, **265**:405-407.
- Foster PL, Trimarchi JM: **Adaptive reversion of a frameshift mutation in Escherichia coli by simple base deletions in homopolymeric runs.** *Science* 1994, **265**:407-409.
- Foster PL: **Population dynamics of a Lac⁻ strain of Escherichia coli during selection for lactose utilization.** *Genetics* 1994, **138**:253-261.
- Hastings PJ, Bull HJ, Klump JR, Rosenberg SM: **Adaptive amplification: an inducible chromosomal instability mechanism.** *Cell* 2000, **103**:723-731.
- Slechta ES, Bunny KL, Kugelberg E, Kofoid E, Andersson DI, Roth JR: **Adaptive mutation: general mutagenesis is not a programmed response to stress but results from rare coamplification of dinB with lac.** *Proc Natl Acad Sci USA* 2003, **100**:12847-12852.
- Slack A, Thornton PC, Magner DB, Rosenberg SM, Hastings PJ: **On the mechanism of gene amplification induced under stress in Escherichia coli.** *PLoS Genetics* 2006, **2**:e48.
- Morag AS, Saveson CJ, Lovett ST: **Expansion of DNA repeats in Escherichia coli: effects of recombination and replication functions.** *J Mol Biol* 1999, **289**:21-27.
- Foster PL: **Adaptive mutation in Escherichia coli.** *J Bacteriol* 2004, **186**:4846-4852.
- Cairns J, Foster PL: **Adaptive reversion of a frameshift mutation in Escherichia coli.** *Genetics* 1991, **128**:695-701.
- Lloyd RG, Low KB: **Homologous recombination.** In *Escherichia coli and Salmonella: Cellular and Molecular Biology* 2nd edition. Edited by: Neidhardt FC. ASM Press; 1996:2236-2255.
- Dutra BE, Suter VA, Lovett ST: **RecA-independent recombination is efficient but limited by exonucleases.** *Proc Natl Acad Sci USA* 2007, **104**:216-221.
- Lloyd RG: **Conjugational recombination in resolvase-deficient ruvC mutants of Escherichia coli K-12 depends on recG.** *J Bacteriol* 1991, **173**:5414-5418.
- Khairnar NP, Kamble VA, Mangoli SH, Apte SK, Misra HS: **Involvement of a periplasmic protein kinase in DNA strand break repair and homologous recombination in Escherichia coli.** *Mol Microbiol* 2007, **65**:294-304.
- Vuong P, Bennion D, Mantei J, Frost D, Misra R: **Analysis of YfgL and YaeT interactions through bioinformatics, mutagenesis, and biochemistry.** *J Bacteriol* 2008, **190**:1507-1517.
- Steiner P, Sauer U: **Overexpression of the ATP-dependent helicase RecG improves resistance to weak organic acids in Escherichia coli.** *Appl Microbiol Biotechnol* 2003, **63**:293-299.
- Zieg J, Maples VR, Kushner SR: **Recombination levels of Escherichia coli K-12 mutants deficient in various replication, recombination, or repair genes.** *J Bacteriol* 1978, **134**:958-966.
- Wang TV, Smith KC: **Inviability of dam recB cells of Escherichia coli is correlated with their inability to repair DNA double-strand breaks produced by mismatch repair.** *J Bacteriol* 1986, **165**:1023-1025.
- Nowosielska A, Marinus MG: **Cisplatin induces DNA double-strand break formation in Escherichia coli dam mutants.** *DNA Repair* 2005, **7**:773-781.
- Lombardo M, Aponyi I, Rosenberg SM: **General stress response regulator RpoS in adaptive mutation and amplification in Escherichia coli.** *Genetics* 2004, **166**:669-680.
- Poteete AR, Fenton AC: **Genetic requirements of phage lambda Red-mediated gene replacement in Escherichia coli K-12.** *J Bacteriol* 2000, **182**:2336-2340.
- Konrad EB: **Method for the isolation of Escherichia coli mutants with enhanced recombination between chromosomal duplications.** *J Bacteriol* 1977, **130**:167-172.
- Blakely GW, Murray NE: **Control of the endonuclease activity of type I restriction-modification systems is required to maintain chromosome integrity following homologous recombination.** *Mol Microbiol* 2006, **60**:883-893.
- Stahl FW, McMillin KD, Stahl MM, Craseman JM, Lam S: **The distribution of crossovers along unreplicated lambda bacteriophage chromosomes.** *Genetics* 1974, **77**:395-408.
- Stahl FW, McMillin KD, Stahl MM, Nozu Y: **An enhancing role for DNA synthesis in formation of bacteriophage lambda recombinants.** *Proc Natl Acad Sci USA* 1972, **69**:3598-3601.
- Stahl MM, Thomason L, Poteete AR, Tarkowski T, Kuzminov A, Stahl FW: **Annealing vs. invasion in phage lambda recombination.** *Genetics* 1997, **147**:961-977.
- Poteete AR: **Involvement of DNA replication in phage lambda Red-mediated homologous recombination.** *Mol Microbiol* 2008, **68**:66-74.
- Court DL, Sawitzke JA, Thomason LC: **Genetic engineering using homologous recombination.** *Annu Rev Genet* 2002, **36**:361-388.
- deMassy B, Fayet O, Kogoma T: **Multiple origin usage for DNA replication in sdrA (rnhA) mutants of Escherichia coli K-12: initiation in the absence of oriC.** *J Mol Biol* 1984, **178**:227-236.
- Boye E, Løbner-Olesen A: **The role of dam methyltransferase in the control of DNA replication in E. coli.** *Cell* 1990, **62**:981-989.
- Kogoma T, Hong X, Caldwell GW, Barnard KG, Asai T: **Requirement of homologous recombination functions for viability of the Escherichia coli cell that lacks RNase HI and exonuclease V activities.** *Biochimie* 1993, **75**:89-99.
- Marinus MG, Morris NR: **Biological function for 6-methyladenine residues in the DNA of Escherichia coli K12.** *J Mol Biol* 1974, **85**:309-322.
- Poteete AR, Fenton AC, Nadkarni A: **Chromosomal duplications and cointegrates generated by the bacteriophage lambda**

- Red system in Escherichia coli K-12.** *BMC Molecular Biology* 2004, **5**:22.
37. Stumpf JD, Poteete AR, Foster PL: **Amplification of lac cannot account for adaptive mutation to Lac+ in Escherichia coli.** *J Bacteriol* 2007, **189**(6):2291-2299.
 38. Rosche WA, Foster PL: **The role of transient hypermutators in adaptive mutation in Escherichia coli.** *Proc Natl Acad Sci USA* 1999, **96**:6862-6867.
 39. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H: **Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection.** *Mol Syst Biol* 2006, **2**:2006.0008.
 40. Murphy KC, Campellone KG, Poteete AR: **PCR-mediated gene replacement in Escherichia coli.** *Gene* 2000, **246**:321-330.
 41. Poteete AR, Fenton AC, Murphy KC: **Roles of RuvC and RecG in phage λ Red-mediated recombination.** *J Bacteriol* 1999, **181**:5402-5408.
 42. Poteete AR, Fenton AC, Wang HR: **Recombination-promoting activity of the bacteriophage λ Rap Protein in Escherichia coli K-12.** *J Bacteriol* 2002, **184**:4626-4629.
 43. Poteete AR: **Modulation of DNA repair and recombination by the bacteriophage λ Orf function in Escherichia coli K-12.** *J Bacteriol* 2004, **186**:2699-2707.
 44. Poteete AR, Rosadini C, Pierre CS: **Gentamicin and other cassettes for chromosomal gene replacement in Escherichia coli.** *Biotechniques* 2006, **41**(3):261-263.
 45. Murphy KC, Campellone KG: **Lambda Red-mediated recombinogenic engineering of enterohemorrhagic and enteropathogenic E. coli.** *BMC Molecular Biology* 2003, **4**:11.
 46. Uhlin BE, Volkert MR, Clark AJ, Sancar A, Rupp WD: **Nucleotide sequence of a recA operator mutation.** *Mol Gen Genet* 1982, **185**:251-254.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

