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Gene Amplification in the *lac* Region of E. coli

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Summary

We have characterized strains of E. coli in which the *lac* region, together with varying amounts of surrounding DNA, is amplified 40 to 200 fold. The amplification events involve regions of 7 to 37 kb and result in a tandem array of repeated units. Restriction digest patterns of DNA from over 100 independent strains reveal that the amplified units are different in each case. Mechanisms of gene duplication and amplification, and the relationship of gene amplification in bacteria to that in eucaryotic cells, are considered.

Introduction

Chromosome rearrangements are involved in gene control, gene amplification, generation of antibody diversity, development, and gene evolution (for review see Schimke 1982a, 1982b). They have also been implicated in the origin of human cancer (Cairns, 1981).

The duplication and amplification of genes have been observed in bacteria in a number of cases (for review see Anderson and Roth, 1977), although only in the *ampC* system of E. coli has DNA sequence analysis been applied (Edlund and Normark, 1981). Because some aspects of gene amplification in bacteria appear strikingly similar to facets of gene amplification in higher cells (see Schimke, 1982a; and Discussion), it is important to understand this process in bacteria, where high resolution genetic methods can be applied. Developing a model system for gene amplification in E. coli may provide valuable insights for understanding gene amplification in higher cells.

We describe the use of the *lac* system of E. coli to study gene amplification. We present the analysis of amplification events involving regions of 7–37 kb, which are increased in copy number 40–200 fold.

Results

The design of the basic experiment aimed at detecting and characterizing amplified *lac* genes is as follows: strains carrying a "leaky" *lac*$^-$ mutation are used to generate Lac$^+$ revertants; DNA from unstable Lac$^+$ revertants is analyzed by restriction endonucleases to identify amplification events; the amplified unit is mapped by the analysis of restriction fragment patterns to trace the origin of the initial duplication event.

The Genetic System

Figures 1 and 2 show the *lac* region of a strain we have used to generate mutants carrying amplified *lac* genes. Figure 1 depicts a deletion that fuses the *Z* gene to the *I* gene (Müller-Hill and Kania, 1974), resulting in a hybrid repressor–β-galactosidase protein which lacks the first 23 residues of β-galactosidase and the last four residues of repressor (Brake et al., 1978). The hybrid protein has normal β-galactosidase activity and is synthesized under the control of the *I* promoter. When the *I* up-promoter mutation *Q* (Müller-Hill et al., 1968) is employed, the fusion strain synthesizes sufficient *lac* enzymes to allow normal growth on lactose. However, if transcription or translation in the *I* portion of the fusion is interrupted, then the Lac$^-$ phenotype results. We have constructed a series of derivatives of the fusion strain depicted in Figure 1, which contain frameshifts or nonsense mutations in the *I* region (Miller and Albertini, 1983). The nonsense mutations result in the termination of translation and the subsequent failure to produce sufficient β-galactosidase to allow growth on lactose.

Certain nonsense sites are located near translation reinitiation sites (for review see Miller, 1974), which allow restoration of varying levels of truncated hybrid repressor–β-galactosidase molecules. In cases where reinitiation is efficient, a leaky Lac$^-$ or even a partial Lac$^+$ phenotype may result. Figure 2 depicts such a situation. When the mutation *X13*, a nonsense mutation at the codon specifying residue 318 in the repressor (Miller et al., 1978), is present in the fusion, translation reinitiation occurs and results in a hybrid β-galactosidase level approximately 8% of that found in the fusion strain in the absence of *X13*.

The reinitiation site is probably the UUG codon at position 319, since the sequence immediately preceding this site, GGGCAA-U-CAGUAG$\overset{319}{\text{UUG}}$, is nearly identical with the sequence preceding the characterized reinitiation site at position 62, GGGCAA-A-CAGUAG$\overset{62}{\text{UUG}}$ (Steege, 1977; Farabaugh, 1978), when an amber codon is at both position 318 and position 61. In this latter case we have demonstrated that reinitiation occurs at the UUG codon at position 62 (Ganem et al., 1973).

The *X13-I-Z* fusion derivative generates a leaky Lac$^-$ phenotype and gives rise to Lac$^+$ colonies at a frequency of 10^{-7} to 10^{-6} in the population, although after several days of incubation on a lactose minimal plate, additional colonies arise out of a hazy lawn. After purification on lactose minimal plates, we streaked pure colonies on nonselective lactose MacConkey indicator medium. Lac$^+$ colonies are red on this medium, whereas Lac$^-$ colonies

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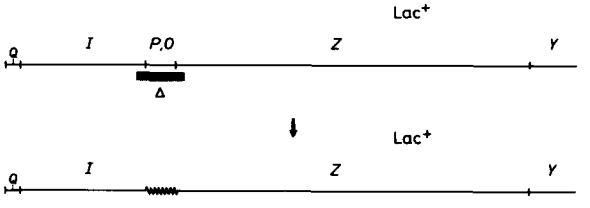

Figure 1. Fusion of *lacZ* to *lacI*

The fusion deletion described by Müller-Hill and Kania, 1974 (see also Brake et al., 1978) is shown. The last four residues of repressor and the first 23 residues of β-galactosidase are missing from the resulting hybrid protein, which is synthesized under the control of the *lacI* promoter.

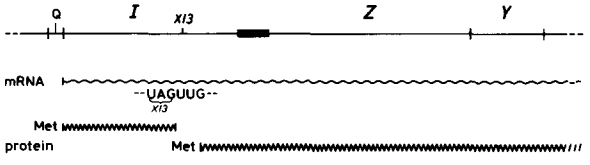

Figure 2. The *lacI-Z* Fusion Carrying Amber Mutation *X13*

The X13 nonsense mutation results in chain termination and the failure to synthesize normal-sized repressor–β-galactosidase hybrid proteins. Low efficiency translation reinitiation at an initiation codon following the *X13* amber (UAG) site allows synthesis of a truncated hybrid protein. The reinitiation codon may be the adjacent UUG codon (see text).

are white. Unstable Lac$^+$ colonies are readily visible on lactose MacConkey plates. Figure 3A shows several unstable Lac$^+$ revertants generated in this manner from the *X13*-fusion strain. The starting strain, shown in the section at the top of the plate, gives pure white colonies on the indicator medium, whereas the unstable revertants are red, but segregate white colonies at a high rate. We can easily transfer the F′*lacproB* episome carrying the *I-Z* fusion and *X13* to other strains deleted for *lacproB*. When we transfer the episomes from the unstable revertants shown in Figure 3A to a *recA*$^-$ strain, the Lac$^+$ character becomes significantly more stable than in the *recA*$^+$ strain. Figure 3B shows the *recA*$^-$ derivatives of previously unstable Lac$^+$ revertants, together with the starting *X13*-fusion Lac$^-$ strain (white colonies). Comparing Figure 3B with Figure 3A shows the increase in stability. When the episomes are transferred back to a *recA*$^+$ strain, the Lac$^+$ character becomes unstable again, as in Figure 3A. Approximately 60% of the Lac$^+$ revertants from the *X13*-fusion strain are unstable. We have not detected unstable revertants in a *recA*$^-$ background.

Biochemical Analysis of Revertants

The properties of the Lac$^+$ revertants described above led us to suspect that amplification of the *lac* region was involved. We therefore purified the DNA from independent revertants and examined the Hinc II restriction endonuclease cleavage patterns. These results are shown in Figure 4. Compared with the control (the Lac$^-$, *X13*-fusion derivative, Figure 4), the pattern of bands seen for the unstable revertants (345–216) is striking. The bands within a single preparation demonstrate molar intensities, indicating equivalent numbers of copies of DNA fragments. The patterns generated by these fragments are relatively simple, and several of the bands, which correspond to *lac* operon sequences (see below), are present in all of the samples. The sizes and numbers of the additional bands vary in each sample. By adding the molecular weights of the bands within a sample, we can estimate the size of the DNA that is amplified. The sizes of the amplified units range from 7 to 37 kb, although the majority are 15 to 20 kb (see below). Although all of 133 unstable revertants tested generated patterns such as those seen in Figure 4, none of over 35 stable revertants tested gave a pattern of bands that was visible in comparison with the control strain.

Determination of Copy Number

To determine if the *lac* sequences were increased in these unstable revertants, DNA digests were electrophoresed as in Figure 4 and then transferred to nitrocellulose filters and hybridized with DNA from pMC1, a plasmid containing the *lacI* region (Calos, 1978). The autoradiograph showed that many more copies of the *lac* region sequences are present in the unstable revertants than in the *X13* parent. To obtain a better estimate of the gene copy number of the *lac* region in the unstable revertants, we diluted a sample of the amplified DNA and compared it to the *X13* nonamplified parent. As Figure 5 shows, comparison of the band intensities of the *X13* DNA and the dilutions of the amplified DNA (after hybridization with pMC1) indicates that the unstable revertants contain between 40 and 80 copies of the *lac* region. Hybridization of similar filters with other regions of the E. coli genome (fragments containing either the chromosomal [*oriC*] or F-episome replication origins or a fragment containing *rpoBC*) did not disclose any increase in DNA copy number.

Mapping the Amplified Units

As a prelude to locating the endpoints of the amplified units, we generated a detailed restriction map of the region surrounding *lac,* which is shown in Figure 6 (see Experimental Procedures for further details).

Initially, we mapped the amplified units using Hinc II. The intense fragments generated by Hinc II digestion of DNA can be aligned with the Hinc II restriction map generated for the plasmid carrying this region (see Figure 6), with the exception of three fragments. The first fragment that does not align with the plasmid-generated fragments is the 574 bp fragment which contains the deletion that fuses the *Z* gene to the *I* gene. In the plasmid these sequences are present on a 789 bp fragment. The second fragment that does not align contains the Tn9 sequence in the pGM11 plasmid, which is not present in the *lac* region of the revertants. The third fragment should be the fusion fragment that contains the junction of the tandem repeats—the 5′ end being contributed by the 3′ end of the amplified unit, and the 3′ end of the fusion fragment being contributed by the 5′ end of the amplified unit.

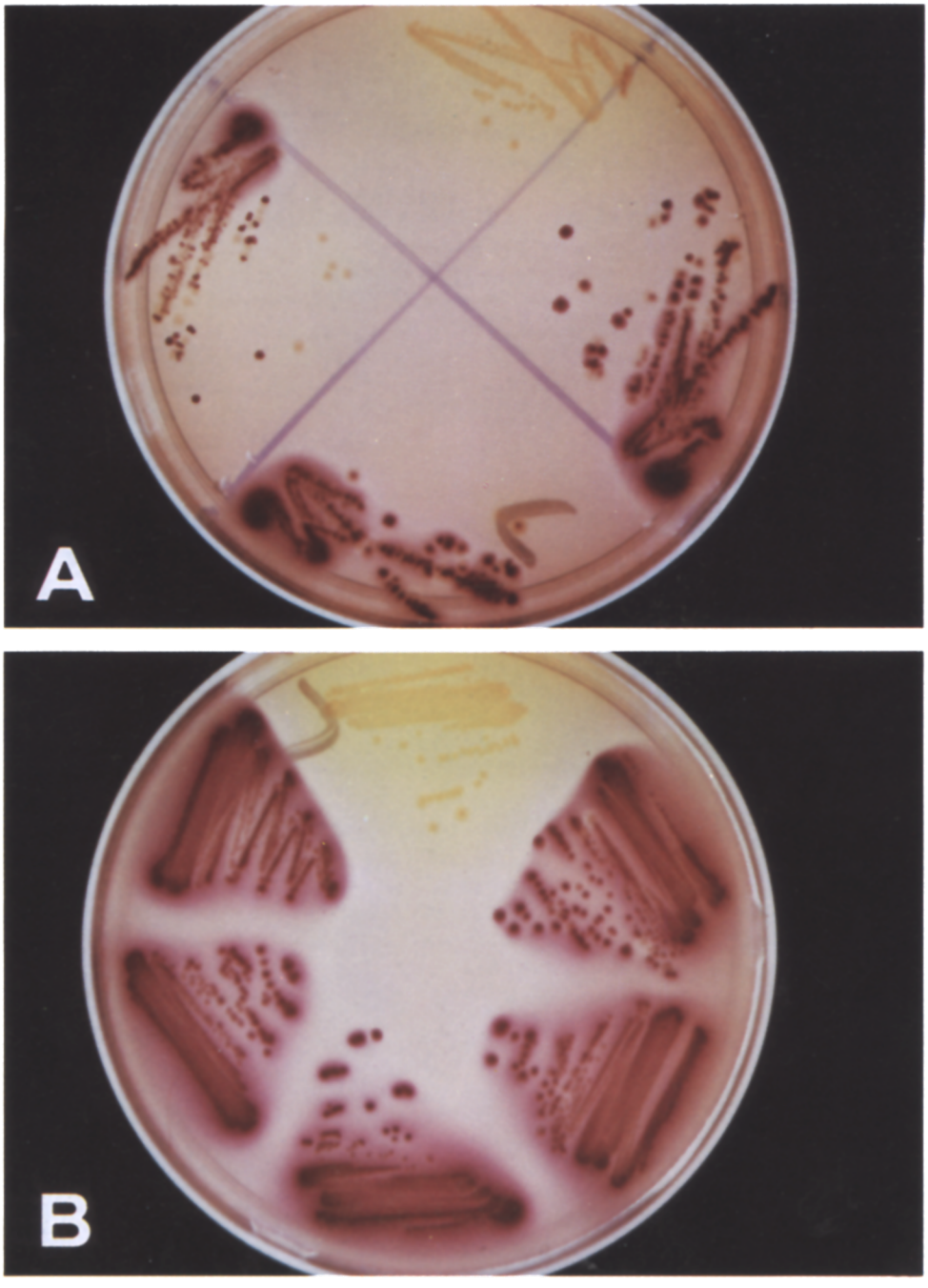

Figure 3. Revertants of the *X13*-Fusion Strain

(A) Unstable revertants of the *X13*-fusion strain. Lactose MacConkey indicator plates (see Miller, 1972) are used to indicate the Lac$^+$ (red) character. The parent strain, carrying the *lac*$^-$ *X13*-fusion, is Lac$^-$ and yields white colonies on this medium (top quadrant). Three independent unstable revertants are indicated in the remaining quadrants. The red colonies are unstable, giving rise to white back-revertants.

(B) The use of a *recA*$^-$ strain stabilizes the *X13*-fusion Lac$^+$ revertants. The top section shows the Lac$^-$ parent, which gives white colonies. The remaining five sections depict revertants that were unstable for the Lac$^+$ (red) character in a *recA*$^+$ background, but are now stable in a *recA*$^-$ background, as can be seen by their failure to give rise to Lac$^-$ (white) back-revertants.

Figures 7 and 8 show the distribution of the endpoints of amplified units for over 100 independent unstable revertants. Fifty of these have both endpoints within the region that is common to both the pGM11 plasmid and the F′*lacproB* episome. These endpoints are depicted in Figure 7. Eighty-five of the amplified units have one or both endpoints outside of the common region. Although there is no obvious hot spot involving both endpoints of the

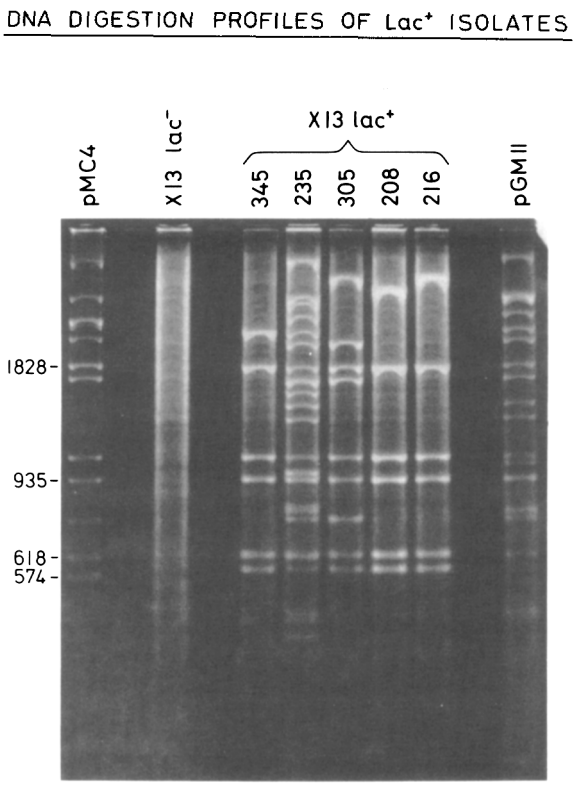

Figure 4. DNA Restriction Endonuclease Digestion Profiles of Unstable Lac$^+$ Revertants

All purified DNAs were prepared as described in Experimental Procedures, incubated with Hinc II restriction endonuclease overnight, electrophoresed on a 1.4% agarose gel, and stained with ethidium bromide. The origins of the DNA samples are indicated above the lanes. Samples 345, 235, 305, 208, and 216 are from the unstable *X13* Lac$^+$ revertants. The pMC4 plasmid carries fragments of 1.83, 0.935, 0.618, and 0.574 kb, which are derived from the *I* and *Z* genes (Calos, 1978). These fragments are visualized in all the unstable revertant DNAs as intensely stained bands. pGM11 is a pBR322 plasmid containing a 27 kb insert of E. coli chromosomal DNA carrying the *lac* region and flanking sequences (Galas et al., 1980). This plasmid does not carry the deletion that resulted in the fusion strain used for this study. As a result, the 0.574 kb *lac* fragment is contained within a 0.78 kb fragment.

same unit, there are regions in which endpoints tend to cluster, as can be seen in Figures 7 and 8. For instance, there are 19 amplified units with one endpoint in the 0.75 kb region on the left side of *lac* as shown in Figure 7, whereas the 1.7 kb and 1.1 kb regions on either side of this 0.75 kb region contain 0 and 2 endpoints, respectively. More detailed restriction mapping should define the degree of clustering within the 0.75 kb fragment and should uncover other clusters of endpoints if they occur. DNA sequence determination of regions containing clusters of endpoints will be of great interest.

Discussion

Previous Studies

One might envision several general models to explain gene amplification. One model, "saltatory replication," postulates

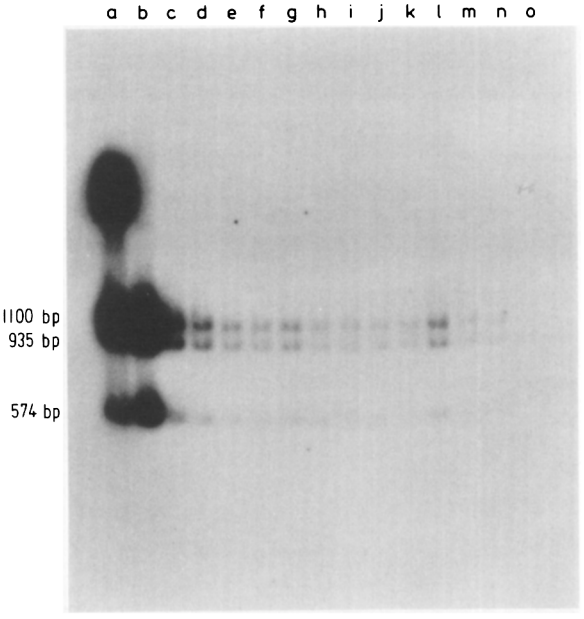

Figure 5. Estimation of the *lac* Region Copy Number

Samples containing decreasing amounts of DNA from an amplified unstable revertant were brought to a constant final concentration by addition of P90C DNA and hybridized with nick-translated pMC1 DNA, as described in Experimental Procedures. Lane (a) contains pMC4 marker DNA; lanes (g) and (l) DNA from the *X13* parent. The remaining lanes contain the following dilutions of DNA from the amplified revertant: (lane b) undiluted; (lane c) 1:20; (lane d) 1:40; (lane e) 1:80; (lane f) 1:100; (lane h) 1:120; (lane i) 1:140; (lane j) 1:160; (lane k) 1:180; (lane m) 1:200; (lane n) 1:300; (lane o) P90C.

that successive rounds of DNA replication in the vicinity of a given gene lead to its amplification. Another model proposes a two-step mechanism. The initial event might be gene duplication, followed by unequal crossing-over to generate amplified sequences (for example see Edlund and Normark, 1981; and Schimke, 1982a). A third model evokes aberrant replication, typified by slipped mispairing to account for deletion formation (Albertini et al., 1982). However, this model might also be applicable to gene amplification. Presently available data do not distinguish among these or other possible hypotheses.

The occurrence of gene duplications is well established in bacteria (Anderson and Roth, 1978; Horiuchi et al., 1963; Rigby et al., 1974; Folk and Berg, 1971; Hill et al., 1977; for review see Anderson and Roth, 1977) and in bacteriophages (Emmons and Thomas, 1975; Emmons et al., 1975). Anderson and Roth (1977, 1981) have demonstrated that duplications of up to a quarter of the Salmonella chromosome occur at large homologous segments, such as the rRNA genes. Such large duplications are dependent on the *recA* system. On the other hand, duplications in the range of 10–20 kb, which do not involve very large homologous segments, appear to be independent of *recA* (Emmons and Thomas, 1975; Emmons et al., 1975; Anderson and Roth, 1978). What is striking about all of these studies is that they indicate that spontaneous duplications occur frequently, on the order of 10^{-5} to 10^{-4} in the

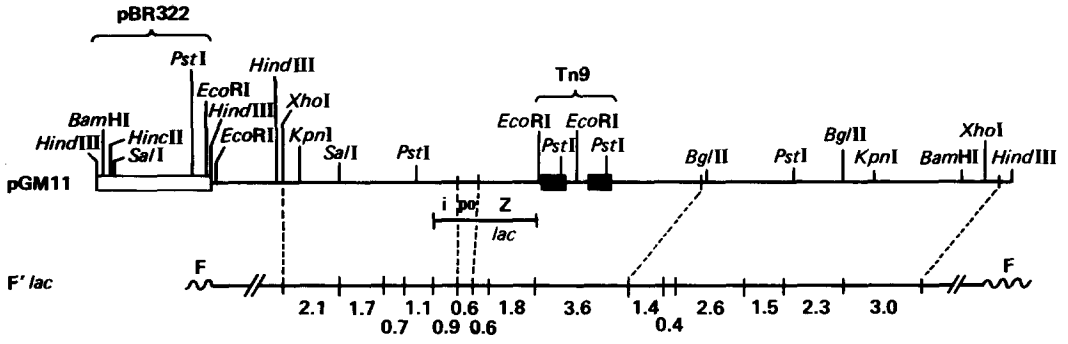

Figure 6. Restriction Map of *lac* Region of E. coli

A map of the *lac* region in E. coli was obtained as described in Experimental Procedures. The top line is a restriction map of pGM11. The bottom line shows the same region mapped in the *lac*⁻ *X13* mutant. This strain has a deletion that fuses the repressor gene to the β-galactosidase gene (Müller-Hill and Kania, 1974), resulting in a shift from the 789 bp fragment to a 574 bp fragment. The numbers on the bottom line refer to the length of the Hinc II fragments.

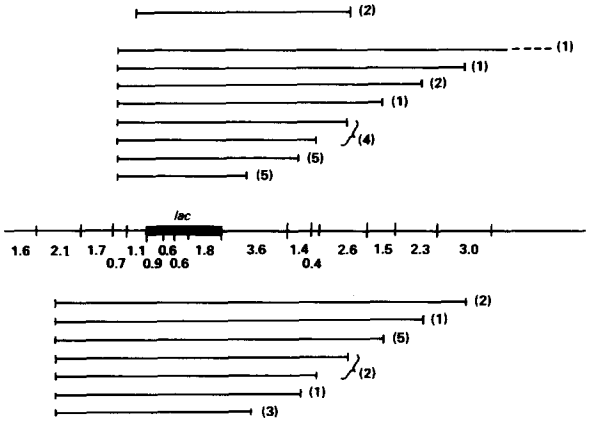

Figure 7. Mapping of the Amplified Units in Independent Unstable Lac⁺ Revertants

DNA from independent Lac⁺ unstable revertants was purified from single colonies as described in Experimental Procedures. This DNA was digested with Hinc II restriction endonuclease and electrophoresed. After staining the gel with ethidium bromide, the intensely staining bands were matched to identically migrating bands generated by the digestion of pGM11. When the next distal fragment was not observed, it was assumed that the endpoint of the amplification unit was located within this fragment. The dashed lines indicate endpoints that lie in unmapped fragments. The numbers in parenthesis indicate the number of independent isolates that fall into each mapping group. Fragments smaller than 400 bp are not shown.

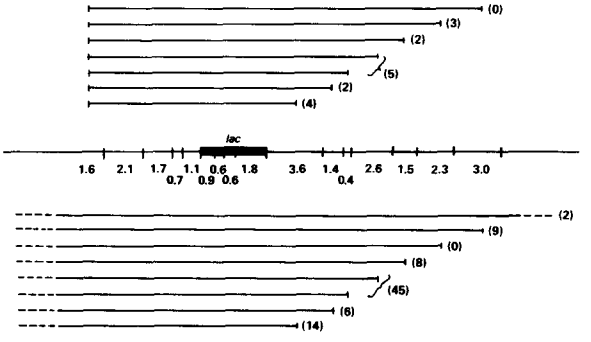

Figure 8. Mapping of the Amplified Units in Additional Independent Unstable Lac⁺ Revertants

Methods used were the same as those described in the legend to Figure 7. In the revertants profiled beneath the Hinc II restriction map of the E. coli lac region the 5′ endpoints fall within an unmapped region.

population for a given region, and at even higher rates when large homologies are involved.

Edlund and Normark (1981) have detected tandem duplications of 10–20 kb at the E. coli chromosomal *ampC* locus, which upon further selection can generate 30–50 tandem copies of the duplicated region. This amplification step is *recA* dependent and probably involves unequal recombination. All eight independently occurring duplication–amplification events analyzed at the restriction fragment level involved different DNA segments. These authors sequenced the juncture points of the amplified unit in one case and found that the original duplication event occurred at a sequence of 12 bp that was repeated on each side of the *ampC* locus, 10 kb apart. Thus the duplication event may represent the reciprocal event of a deletion at a short repeated sequence, as we have found frequently in the *lacI* system (Farabaugh et al., 1978; Albertini et al., 1982).

Recent reports have also described a 30-fold amplification of chromosomally integrated plasmid sequences surrounding the *amp* or *tet* genes in E. coli (Gutterson and Koshland, 1983), as well as a 500-fold amplification of a 10.5 kb sequence in Streptomyces fradiae (Fishman and Hershberger, 1983).

Amplification in *lac*

We report a system for detecting amplification events as revertants of certain Lac⁻ mutants. We analyzed large numbers of independent events and characterized over 100 examples of unstable revertants with 40–200 copies of the *lac* region. Most unstable revertants amplified a 10–30 kb region, and the majority of the amplified units are different, as shown by restriction digestion patterns. We detected the amplification events at a level close to 10^{-6} in the population. Figure 9 depicts an amplified *lac* region in schematic form.

Much information can be gained from the inspection of

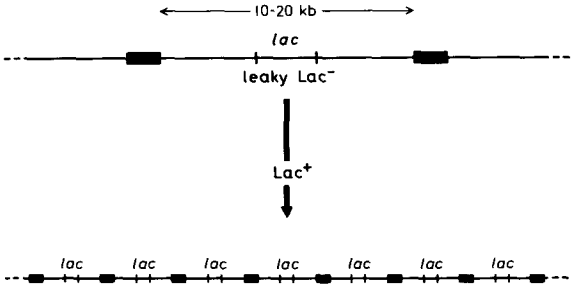

Figure 9. Schematic Representation of Amplification of the *lac* Region

the patterns generated by endonuclease digestion of DNA from the unstable Lac$^+$ revertants (Figure 4). The fact that the bands are distinct and present at molar intensity indicates that the unit of amplification in each revertant is defined and constant. If the endpoints of the amplification unit varied within a single revertant, one would see some bands of greater intensity than others. Using the same information, it can be seen that all but one of the intense fragments generated in the unstable Lac$^+$ revertants correspond to the fragments generated by digestion of the plasmid containing this region of the E. coli chromosome. This unique "fusion" fragment contains sequences of DNA from both ends of the amplified unit as well as the junction where the tandem units abut. When this fusion fragment is excised, nick-translated, and hybridized back to a digest of unamplified DNA, the two expected fragments are visualized (data not shown). The existence of only one fusion fragment indicates that the tandem repeat is in a direct orientation. Tandem duplications in an inverted orientation would generate two fusion fragments.

Pathways of Rearrangement

The work described above raises a myriad of questions. What is the involvement of the *recA* system in the formation of large duplications or amplifications? The increased stability of the Lac$^+$ character in a *recA*$^-$ strain argues that unequal recombination is involved in the reduction of copy number. Perhaps an increase in copy number can occur by the same mechanism if a tandemly duplicated region is present. Although we did not detect amplification events in a *recA*$^-$ strain in the *X13*-fusion system, we cannot say whether the *recA* dependence is due only to subsequent amplification following duplication, or whether it results from reduced frequency of an initial duplication as well. The separation of each stage of the amplification process is required in order to evaluate the role of the *recA* system.

Are short homologous sequences required for generating large tandem duplications or amplifications? We recovered amplified units that ranged from 7 to 37 kb in length. Most of the amplified units have unique endpoints, as judged by restriction analysis. From the large number of different endpoints of the amplified units in the *lac* region, it is unlikely that most of the original duplications occurred at a repeated sequence as large as 12 bp, as in the case of *ampC* (Edlund and Normark, 1981). Therefore, either shorter repeats are used or perhaps many of the duplications do not involve repeated sequences at all.

Is there a special mechanism for generating large duplications or amplifications in bacteria? Anderson and Roth (1981) estimated that as much as 3% of a growing population contains tandem duplications. Indeed, they suggest that many such duplications confer a selective advantage. Perhaps a system responsible for these rearrangements is inducible under growth-limiting conditions, such as those resulting from the selective pressures exerted in the experiments cited above.

Does the generation of highly amplified genes follow simply from tandem duplications by unequal crossing-over (see above), or are there other pathways involved? How do different mutagens affect amplification? Is amplification dependent on the position of the *lac* region (e.g., the location on the episome or chromosome)? These questions will be addressed with the amplification detection system we have described above.

Amplification in Eucaryotes

Many examples of gene amplification in eucaryotes have been documented. In some cases, amplification is developmentally regulated. For instance, Brown and Dawid (1968) have shown that Xenopus oocytes amplify ribosomal genes in a controlled manner. In Drosophila, a set of chorion genes in the ovaries is amplified 25-fold during egg laying (Spradling and Mahowald, 1980). In other cases, many mutational studies have inadvertently selected for gene amplification. For example, Alt et al. (1978) discovered that one mechanism for resistance to methotrexate was amplification of the gene for the targeted enzyme, dihydrofolate reductase. Similar examples have been reported with other agents that can cause metabolic stress (Wahl et al., 1979; Beach and Palmiter, 1981; Brennand et al., 1982; Coderre et al., 1983). Thus this type of gene amplification may be a more general phenomenon than has previously been appreciated.

The best studied eucaryotic examples, amplification of the dihydrofolate reductase gene or the CAD gene, show striking similarities to *lac* amplification in E. coli. Such amplifications involve relatively large segments of DNA, may consist of many tandem copies which may be intrachromosomal, and occur at low but detectable frequencies. Further structural information regarding eucaryotic gene amplification is difficult to obtain (e.g., see Schilling et al., 1982, and Ardeshir et al., 1983). With the *lac* system, we can easily determine the size and orientation of the amplified units. Currently, bacterial gene amplification allows for higher resolution of the events and structures involved.

Experiments have shown that carcinogens can activate amplification events in mammalian cells (Tlsty et al., 1982; Lavi, 1982). It will be of interest to study the effect of carcinogens on the activation of gene amplification in bacteria and to relate the mechanisms deduced in bacterial systems to the comparable processes in higher cells. The study outlined here is the beginning of an investigation of

genetic rearrangements of large regions of the chromosome. The possible importance of rearrangements in the origin of human cancer has been considered (Cairns, 1981).

Experimental Procedures

Detection of Lac$^+$ Revertants

Overnight cultures were plated on lactose minimal medium (media as described in Miller, 1972), and colonies were picked after 48 hr. After purification on minimal lactose plates, single colonies were streaked on lactose MacConkey plates to determine what proportion had a stable or unstable phenotype. One unstable revertant was chosen from the minimal lactose plate corresponding to each separate overnight culture for further analysis.

Rapid Isolation of DNA from a Single Colony

DNA was prepared according to the procedure outlined in Davis et al. (1980). A single colony was taken from a minimal lactose plate, transferred to 1 ml of 50 mM Tris (pH 8.5), 50 mM EDTA, 15% sucrose, and thoroughly suspended. The cells were pelleted and resuspended in 0.5 ml of the above solution with an addition of 1 mg/ml lysozyme (freshly prepared). Samples were incubated at room temperature for 10 min, and about 10 μl of 10% SDS was added. Tubes were gently inverted twice and heated at 70°C for 5 min. Immediately afterward, 50 μl of 5 M KOAc was added and the tubes were placed on ice for at least 30 min. The precipitate was sedimented in a microfuge for 15 min, and the supernatant was transferred to a clean microfuge tube. The sample was precipitated with ethanol and resuspended in 10 mM Tris (pH 7.5), 1 mM EDTA, and 10 μg/ml RNAase A and incubated at 37°C for 2 hr. After a second ethanol precipitation, the samples were digested with restriction endonucleases according to the conditions indicated by the supplier (New England BioLabs).

Preparation of Large Quantities of DNA

Purified DNA was prepared from the parent *I-Z* fusion strain carrying the *X13* mutation and several of the unstable Lac$^+$ revertants. Bacteria were scraped off minimal lactose plates, lysed with lysozyme (1 mg/ml) and Sarcosyl (1%), incubated with RNAase (10 μg/ml) and protease (50 μg/ml), and extracted twice with buffer-saturated phenol. The DNA was precipitated with ethanol, centrifuged in CsCl overnight, and reprecipitated in ethanol. Samples were digested with restriction endonucleases according to the conditions suggested by the supplier.

Agarose and Acrylamide Gels

Restriction fragments generated from the digestion of DNA from the unstable revertants were electrophoresed on 1.4% agarose gels (agarose from Sigma). Small fragments were analyzed on 8% acrylamide gels. Both types of gels were electrophoresed in Tris, borate, EDTA buffer (Davis et al., 1980).

Transfer of DNA to Nitrocellulose and Hybridization to Labeled DNA Probes

The restricted DNA was transferred from the agarose gel to two nitrocellulose filters by a bidirectional blotting procedure (Smith and Summers, 1980) and after being baked onto the filter was hybridized with ^{32}P-labeled DNA. Prior to hybridization, the filters were presoaked at 65°C in a solution containing 5× SSC or SSPE, 100 μg/ml of sonicated denatured calf-thymus DNA, 1× Denhardt's solution and 0.3% SDS. The solution was replaced after 2–3 hr with the same solution containing the radioactive probe for hybridization.

Nick translation of the DNA probes was according to Rigby et al., 1977. α-^{32}P-dATP from Amersham was used (3000 Ci/mmole). After hybridization for 12–24 hr at 65°C the filters were washed for a total of 4 hr in four changes of 2× SSPE, 0.2% SDS, and finally for 10 min in 10 mM Tris (pH 7.5). The filters were air dried and exposed to X-ray film at −70°C.

Quantitation of DNA Amplification

Purified DNA was prepared from the *lacI(X13)-Z* fusion parent strain, from a strain deleted for *lac* (P90C), and from unstable Lac$^+$ revertants of the *X13*-fusion parent, as described above. A constant quantity of DNA (1 μg) was used for each lane. Samples containing decreasing amounts of DNA from an amplified unstable revertant were brought to a final concentration of 1 μg by addition of the appropriate aliquot of P90C DNA. The DNA was digested with Hinc II restriction endonuclease overnight, transferred to two nitrocellulose filters (Smith and Summers, 1980), and used for hybridization with ^{32}P-labeled pMC1 and pJC720. The pMC1 plasmid carries a 2800 bp fragment of the E. coli chromosome that contains the *lacI* gene and the beginning of the *Z* gene (Calos, 1978). When this plasmid is hybridized to Hinc II–digested DNA containing *lac* sequences from the fusion strain, three fragments are visualized by autoradiography (1.1, 0.935, and 0.574 kb). The second filter was hybridized to pJC720 (a gift from P. Prentki), which contains an E. coli chromosomal fragment carrying the *rpoBC* region (Collins et al., 1976).

Similar filters were also hybridized with nick-translated DNA fragments bearing the episomal origin of replication and the chromosomal origin of replication (both gifts from P. Prentki) (data not shown).

Extraction of DNA from Acrylamide Gels

The desired DNA fragment was visualized with ethidium bromide and was cut out of a 6% preparative acrylamide gel and mashed with a spatula. The mashed fragments were placed in a blue Eppendorf tip that had been sealed with hot forceps at the tip and plugged with siliconized glass wool. The gel fragments were incubated at 42°C overnight in 0.7 ml of solution X: 0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1% SDS, and 0.1 mM EDTA. Each tip was placed in a small glass tube (Pyrex, 10 mm × 75 mm), and following incubation the ends were cut off and the set was centrifuged at 3000 rpm for 5 min. The gel fragments were rinsed with 0.2 ml of solution X and centrifuged again. The DNA was precipitated with ethanol twice and was used for end labeling or nick translation.

Mapping of the DNA Region Flanking the Lactose Operon

A large plasmid (pGM11) that carries 27 kb of the E. coli genome, including the *lac* region and a Tn9 element (Galas et al., 1980), was used to map the Hinc II restriction endonuclease sites around the lactose operon. Mapping was accomplished by two methods. First, the sites of infrequently cutting restriction endonucleases were determined by single and simultaneous digestions with the enzymes shown in Figure 6. This produced the map illustrated in Figure 6. These sites were localized by hybridization to subclones of the plasmid. The 27 kb insert was separated from the vector by Hind III digestion and then cut in the center at the Eco RI site, located at the end of the *Z* gene. The two genomic fragments (containing the 3′-flanking sequences of *lac* in one and the 5′-flanking sequences in the other) were labeled by nick translation and used for Southern hybridizations to Hinc II–digested pGM11 plasmid DNA to assign fragments to the 3′- or 5′-flanking regions.

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