RECIPIENT GENE DUPLICATION DURING GENERALIZED TRANSDUCTION

M. STODOLSKY

Loyola University Stritch School of Medicine, Maywood, Illinois 60153

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ABSTRACT

An Hfr1 ΔproA-lac deletion recipient, ΔproA-lac-F-purE-, has been utilized in a study of the origins of duplications formed during chromosome fragment integration. Among the Pro-Lac+ transductants, some have duplications spanning the F locus. These transductants are, or segregate, strains with F' episomes carrying genes of the duplication. Some of the duplications include purE+, a gene which is not coinherit with lac+ during bacteriophage P1-mediated transduction. Thus recipient genes have been duplicated during recombinant formation. Crossing-over models including replication steps provide a basis for explaining the duplication process.

HETEROZYGOTES are sometimes formed during generalized transduction of bacterial genes (Lennox 1955; Markovitz 1964). Their unstable genetic elements are now recognized to be components of cis-oriented chromosomal duplications (Hill et al. 1969). This study is concerned with the origins of genes in duplications so formed. In a previous study, lac+ transduction from Escherichia coli proA+ lac+ to recipients with proA-lac deletions was characterized (Stodolsky, Rae and Mullenbach 1972). This system provides an absolute selection for partial diploids, because the absorbed lac+ fragments are too short to be integrated by a replacement of the deletion. Transductants have genetically unstable lac+ units, designated lacV, which are integrated in tandem with the deletion. The formation of their recombinant chromosomes can be depicted as proceeding through some sequence of crossing over and fusion. Plausible candidates for the critical stabilizing event of fusion (end-to-end joining of genetically heterologous DNA’s) are: a base pairing of “accidentally” complementary DNA ends; the priming of DNA synthesis (Goulian 1968; Stodolsky 1973a); and ligation (Sgarrella, Van de Sande and Khorana 1970).

This study was motivated by the following observations. The ΔproA-lac/lacV heterozygotes are diverse in the stability of their lac+ genes, ranging from quasi-stable types to some which are almost too unstable to maintain even under selective conditions. It is plausible to attribute differences in heterozygote stability to the length of their duplicated gene segments. The longer a duplication, the higher the probability (per generation) that crossing over within units of the duplication will occur; if the crossover is between distinct components of the duplication, a haploid segregant is formed. Accepting this working interpreta-
tion, it seemed possible that the more unstable heterozygotes might have duplications longer than the chromosome of the ancestral lac fragment. That is, recipient genes which were not represented in the absorbed lac+ fragment might be included in the duplication of the recombinant ΔproA-lac/lacV chromosome. To simply test this suspicion a novel trick has been employed in this study. The difficult task of lacV gene content analysis has been replaced by a simpler but equivalent F'lac gene content analysis. It appears that duplications of recipient genes do occur during the generalized transduction. A molecular basis for the duplication process is offered. A preliminary report of the results has been presented (Stodolsky 1972).

### TABLE 1

E. coli K12 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characters</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>x316</td>
<td>F- prototroph</td>
<td>R. Curtiss III</td>
</tr>
<tr>
<td>x316</td>
<td>F-Δ proA-lac deletion</td>
<td>R. Curtiss III, from x316</td>
</tr>
<tr>
<td>W3747†</td>
<td>met/F13 argF+ lac+ proC+ lon+ (capR)+ purE+</td>
<td>W. Epstein</td>
</tr>
<tr>
<td>SC1500</td>
<td>ΔF111 (proA-argF-lac deletion X111)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>his strA metB argl</td>
<td></td>
</tr>
<tr>
<td>SC1401</td>
<td>ΔproA-lac/F13 lac+</td>
<td>W3747 × x354</td>
</tr>
<tr>
<td>SC1402</td>
<td>Hfr13 ΔproA-lac</td>
<td>segregant of SC1401</td>
</tr>
<tr>
<td>SC1405</td>
<td>ΔF-ΔproA-lac strA‡</td>
<td>spontaneous mutant of x354</td>
</tr>
<tr>
<td>SC0622</td>
<td>F-Δ111 recA1</td>
<td>Stodolsky et al. (1972)</td>
</tr>
<tr>
<td>MC100</td>
<td>leu lacY proC purE galK trp strA</td>
<td>A. Markovitz, x156 of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. Curtiss III</td>
</tr>
<tr>
<td>MC102</td>
<td>leu lacY capR9 purE galK trp strA</td>
<td>A. Markovitz</td>
</tr>
<tr>
<td>KL1699</td>
<td>HfrKL16 recA1</td>
<td>W. Epstein</td>
</tr>
<tr>
<td>SC0701</td>
<td>HfrKL16 recA1 mal (λ)</td>
<td>Spontaneous mutant of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KL1699, resistant to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bacteriophage A and Mal-</td>
</tr>
<tr>
<td>SC1416</td>
<td>ΔproA-lac/F13 lac+</td>
<td>Trimetaphrom-resistant§</td>
</tr>
<tr>
<td>SC1426</td>
<td>ΔproA-lac thyA/F6</td>
<td>mutant of SC1416</td>
</tr>
<tr>
<td>SC1427</td>
<td>ΔproA-lac recA1/F6</td>
<td>SC0701 × SC1416†</td>
</tr>
<tr>
<td>SC8305</td>
<td>Hfr13 ΔproA-lac purE</td>
<td>SC1402 × MC102</td>
</tr>
</tbody>
</table>

* x354 was initially given a proA+ proK-lac designation by R. Curtiss III. However, I have found that x354 has an amber suppressor, which probably led to its mis-characterization following introduction of pro amber alleles. Recent deletion mapping and complementation tests demonstrate that: there is no essential "proB+" marker between argF and lac (contrary to the current E. coli map); x354 lacks proA; the initial proB and proA distinctions (based solely on conjugal cross data) are not reliable predictors of complementation behavior (Ackerman and Epstein, personal communication). Complementing pro alleles (exclusive of proC) are very tightly linked (Condamine 1971; Stodolsky 1973b). For these reasons proB is not utilized herein, and proA is utilized only as a general chromosomal locus designation.

† Lon and capR are alternative designations for a locus pleiotropic for control of radiation sensitivity and capsular polysaccharide synthesis.

‡ StrA strains are resistant to 100 μg/ml of streptomycin (strR).

§ Selected on minimal agar containing 10 μg/ml Trimethoprim (Burroughs, Wellcome and Co.) and 200 μg/ml thymine.

† SC1426 was converted into an F- phenocopy by growth at 25° prior to a 30-minute mating. SC1427 was identified as an exconjugant with sensitivity to ultraviolet light, comparable to SC0701 recA1 mal−.
MATERIALS AND METHODS

Media—Synthetic and broth media have been described (STODOLSKY, RAE and MULLENBACH 1972). The minimal media do not contain Ca++, which is required for bacteriophage P1 absorption. Thus P1 does not propagate on the transduction plates. On EMB lactose indicator agar, Lac- colonies or sectors are pink, while lactose catabolizing bacteria stain purple to black. Sectors are most readily resolved after 18–24 hrs at 37°C.

Bacteriophage—A rapidly absorbing (g character) prophage repressor negative P1 mutant, P1cl g, was obtained from N. FRANKLIN and used in all transductions.

Bacteria—E. coli K12 strains utilized are described in Table 1 and marker loci are depicted in Figure 1. Shigella dysenteriae Sh was utilized for P1 titration.

Strain SC8306 ΔproA-lac purE strA was constructed in the following way. SC1402 Hfr13 ΔproA-lac leu+ cap+ pur+ trp+ str8 was mated with MC102 leu- lacY capR9 purE trp- strA. Conjugal pair disruptions were performed at 30, 60, 90 and 120 minutes. The treated samples were diluted 1000-fold and grown for an hour more to permit the segregation of StrR recombinants from sensitive strA/str+ exconjugants. Leu+ Trp+ StrR-resistant recombinants were selected on minimal agar plates which were permissive for other characters. Cap+ colonies were recognized by their mucoid character. Pro- Pur- colonies were detected by replica plating to suitable minimal agars. The desired recombinant could most simply have arisen through crossovers which integrated a leu+ ΔproA-lac-F-cap- segment of the SC1402 chromosome into the MC102 chromosome, with the recipient trp- allele being replaced by other crossover events. Pro- recombinants were only obtained from the sample which was disrupted 120 minutes after mating was initiated. This observation shows that ΔproA-lac is a late marker of SC1402, and justifies (together with the fact that proC+ is transferred early to MC100 proC strA) the ΔproA-lac-F-proC+ linkage assignment for SC1402.

Transduction—P1cl g was absorbed for 30 minutes at 25°C to bacteria grown in L broth. Infected bacteria were washed in saline by centrifugation and plated on minimal agars.

Conjugal crosses—Crosses in broth (STODOLSKY, RAE and MULLENBACH 1972) and matings by replica plating (PITTARD 1965) have been described previously.

Purifications—Some P1cl g does survive during incubation on the selection plates, and is carried along during transfer of transductant colonies. For this reason it is necessary to disperse...
transductants in (Ca++-free) saline to avoid P1coIg infections. New strains were purified by at least two subcultures on selection or indicator agars.

RESULTS

The experimental design: A primary problem in undertaking this study was the design of a simple method for determining the extent of duplications. This problem is trivial when a duplication is heterozygous for many of its genes. It then suffices to identify the various segregant types. But when it is suspected that part of a duplication may be homozygous (being derived only from recipient genes) a more general method is necessary. The following stratagem was motivated by Pittard’s (1965) observation that F excision from an Hfr chromosome is stimulated when recombination occurs around the origin of the integrated F factor.

Consider a duplication in which F is within only one of the genetic repeats (the duplication is “heterozygous” for F). Such duplications will sometimes be formed in transduction during lac+proC+ fragment addition to the ΔproA-lac-F-proC+ chromosome of an Hfrl3 recipient (Figure 2). The transductant is equivalent to a ΔproA-lac-proC+/F' lac+ proC+ heterozygote, except that F' will be in the integrated state. Episomes carrying lac+ and the recombinant’s duplicated genes will sometimes be excised by legitimate (gene-linkage-conserving) crossovers (Campbell 1962).

In the case of an F' formed within an Hfrl3 population, lac+ is a distal marker of the episome (Figure 1). Genes transferred from the F' population with lac+ in a short mating are on the episome and can be detected through transfers to suit-
able multiply auxotrophic recipients. Thus the characterization of F′lacS of transductant populations provides a simple and rapid method for assaying the gene content of the original duplication.

An alternative but more laborious procedure is the identification of the repeat point or joint (jnt) of the duplication through transduction crossovers (Hill, Schiffer and Berg 1969).

**Construction of the recipient:** To construct the required Hfr recipient for transduction the F′3lac episome of W3747 metB/F′13lac+ proC+ was introduced into x354 ΔproA-lac and SC1401 ΔproA-lac/F′13 was selected. To obtain the desired Hfr segregant, the F′ population was propagated at 37° in L broth. Under these conditions infection of F−ΔproA-lac segregants with F′13 is rampant. Consequently the Lac− segregants which accumulate are Hfr13 ΔproA-lac strains. F′13 could not propagate as an episome in these segregants, even if absorbed, because of competition for F membrane maintenance sites. SC1402 Hfr13 ΔproA-lac was picked as a Lac− segregant of SC1401 ΔproA-lac/F′13. It transfers proC+ as an early marker. It transfers ΔproA-lac as a late marker, as found during the construction of SC8306 Hfr13 ΔproA-lac purE strR (see Materials and Methods).

**Transductions:** A prophage repressor-defective bacteriophage P1, P1cl g, was used as the vector in all transductions and m.o.i. of 0.3 were employed. P1Δlac and P1Δpro lysogens are sometimes formed in recipients of P1cl+ and transducing particles (Luria, Adams and Ting 1960; Rae and Studolsky 1973; Studolsky 1973b). The use of a P1cl vector has barred their formation in this study.

Gross characteristics of the transduction system are presented in Table 2. Lac+ transduction of x354 ΔproA-lac is about 10-fold less efficient than transduction of the x474 lacY recipient. In the latter strain lac+ integration can proceed by a simple replacement of the lacY allele. A comparison of lac+ transduction to x354 F− ΔproA-lac and SC1402 Hfr13 ΔproA-lac suggests that the presence of F in the chromosomal region in which the lac+ fragments must be integrated does somewhat diminish the efficiency of lac+ integration by addition. Pro+ gene addition to these two strains proceeds at identical frequencies.

**F′ strain formation in transductants of SC1402 Hfr13 ΔproA-lacstrR:** To simply identify F′ strains among transductants, a qualitative test distinguishing early and late marker transfer was employed. Markers transferred after strR from

| Table 2 |
| Transduction of lac+ and proA+ from x316 pro+lac+pur+ to ΔproA-lac recipients |

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Lac+</th>
<th>Transductants per infectious virion for:</th>
<th>Lac+Pro+</th>
<th>Lac+pur+</th>
</tr>
</thead>
<tbody>
<tr>
<td>x354 F− ΔproA-lac</td>
<td>9×10−8</td>
<td>2×10−7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SC1402 Hfr13 ΔproA-lac</td>
<td>3×10−8</td>
<td>2×10−7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SC8306 Hfr13 ΔproA-lac purE</td>
<td>2×10−8</td>
<td>—</td>
<td>2×10−6</td>
<td>—</td>
</tr>
<tr>
<td>x474 F− proA lacY purE</td>
<td>10−8</td>
<td>10−8</td>
<td>3×10−6</td>
<td>-<em>&lt;3×10−9</em></td>
</tr>
</tbody>
</table>

* Transductants and controls (1010 of each) were plated on 2 ft. square plastic dishes, to avoid exhaustive catabolism of supplements by concentrated bacteria.
Hfr13 str\(^\text{R}\) donors rarely appear in str\(^\text{R}\) exconjugant lines. Str\(^\text{S}\) is dominant in new str\(^\text{R}/\text{str}\text{S}\) exconjugants, and recipients of markers distal to str\(^\text{S}\) are killed when the selection is applied prior to haploidization. Lac\(^+\) or pro\(^+\) genes added to the Hfr13 \(\Delta\text{proA-lac}\) chromosome next to the deletion would be transferred as late markers. However F\(^{+}\) segregants of transductants would transfer out F\(^{+}\) independently of the host str\(^\text{S}\) chromosome, and str\(^\text{R}\)/F\(^{+}\) exconjugants could thereby be obtained.

Transductants were characterized in parallel using a plate mating technique (Pittard 1965). Pro\(^+\) and Lac\(^+\) transductants were spread in patches on the original selection agars and replicas of these master plates were used in conjugal crosses. Crosses were initiated by replicating transductant patches onto SC1504 \(\Delta\text{proA-lacstr}\text{R}\) recipients on streptomycin-containing agar. SC1401 \(\Delta\text{proA-lac}/\text{F}\text{'13 lac}^{+}\) served as an F\(^{+}\) control.

Of 19 Lac\(^+\) transductants 10 were identified as good lac\(^+\) donors to SC1500 \(\Delta\text{m11 his-met-argI str}\text{R}\). Their Lac\(^+\) exconjugants were subcloned on EMB lactose agar and replicated onto lawns of SC0622 \(\Delta\text{m11 recA1 his}^{+}\text{ met}^{+}\text{ argI}^{+}\) on lactose proline agar. Auxotrophies of the donor served as the counterselection. More than 50% of each population of subcloned exconjugants transferred lac\(^+\) to the recombination-deficient recipients. The successive transfer of lac\(^+\) from the transductants to SC1500 and SC0622 establishes that the transductants have fertile episomes.

If the proposed explanation of F\(^{+}\)lac formation within the transductants (Figure 2) is valid, then F\(^{+}\)pro strains must not be present among Pro\(^+\) transductants of SC1402 Hfr13 \(\Delta\text{proA-lac}\). The lack of coinheritance of lac\(^+\) with pro\(^+\) (Table 2) shows that the proV elements in recombinant chromosomes (\(-\text{proV-}\Delta\text{proA-lac.F-proC}^{+}\)) do not genetically span the deletion. Hense the proV elements do not span the F locus as well, and F\(^{+}\) pro formation cannot proceed through the legitimate excision scheme of Figure 2. Using the plate mating technique, 20 Pro\(^+\) transductants were crossed with SC1405 \(\Delta\text{proA-lacstr}\text{R}\). None of the 20 transductants are early pro\(^+\) donors and therefore do not contain F\(^{+}\)pro\(^+\) episomes.

The lack of F\(^{+}\)pro formation in the Pro\(^+\) transductants and the frequent F\(^{+}\)lac formation in the Lac\(^+\) transductants provides strong support for the F\(^{+}\) genesis scheme of Figure 2.

To justify the use of F\(^{+}\)lac's to determine the gene content of a transductant's duplication, it was first essential to show that the same F\(^{+}\)lac is formed in distinct lac\(^+\) excisions from the chromosome of a particular Hfr13 \(\Delta\text{proA-lac}\text{V}\) transductant. That is, it is conceivable that the recombinations mediating F\(^{+}\)lac\(^+\) excisions might more commonly be genetically aberrant than genetically legitimate. Then distinct F\(^{+}\)lacs would arise within a single Hfr13 \(\Delta\text{proA-lac}\text{V}\) transductant line, and F\(^{+}\)lac analysis could not be substituted for lac\(^{\text{V}}\) gene content analysis. To examine this possibility, strains with lac\(^+\) still in the integrated state were sought among the 10 transductant populations which yielded positive results in the patch tests. Six transductants appeared to be predominantly in the F\(^{+}\) state. They were relatively stable for lac\(^+\) and poor conjugal trp\(^+\) donors. In populations of each of the remaining four transductants, two colony types could
TABLE 3
Conjugal transfer from Lac+ transductants of SC1402 Hfr13 Δpro-lac to MC100 lacY trp strR

<table>
<thead>
<tr>
<th>Donor</th>
<th>Exconjugants per donor for*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC1402 Hfr13 Δpro-lac</td>
<td>Lac+</td>
</tr>
<tr>
<td>Transductant 4†</td>
<td>0.2</td>
</tr>
<tr>
<td>Hfr13 Δpro-lac/lacV</td>
<td>0.3</td>
</tr>
<tr>
<td>Δpro-lac/F lac+proC+</td>
<td>0.8</td>
</tr>
<tr>
<td>SC1416 Δpro-lac rec+/F lac+purE+</td>
<td>0.7</td>
</tr>
<tr>
<td>SC1427 Δpro-lac recA1/F lac+purE+</td>
<td>0.5† &lt; 10⁻⁴</td>
</tr>
</tbody>
</table>

* Conjugal pairs were diluted into saline, after 40 minutes at 37° and vortexed (Vortex Junior Mixer) for one minute before plating.
† Transductants 5, 16, and 18 gave similar results.
‡ Lac+ exconjugants were Pur+.

be distinguished on EMB lactose indicator agar: dark colonies which rarely exhibited a Lac- sector, and more lightly staining and more frequently variegated Lac+ colonies. Variegated colony-forming types segregated the dark colony-forming ones. The variegated to dark colony transition probably reflects the transition of lac+ from an integrated state in Hfr13 Δpro-lac/lacV transductants to an autonomous state in Δpro-lac/F lac+ segregants (Figure 2); variegated colonies (one tested for each transductant) were more effective conjugal trp+ donors than their dark segregants (Table 3). To determine if the F' strains derived from a single more unstable predecessor were identical, five independent segregants from transductant #4 were compared by the tests described below. Each contained a F' lac+ proC+ episome. This is the longest episome present among the transductants for which an Hfr Δpro-lac/lacV predecessor was detected. Since identical episomes are being formed within a single Hfr13 Δpro-lac/lacV transductant line, a substitution of F lac gene content analysis for lacV gene content analysis is acceptable.

F' lac genes: Exconjugants formed through F' lac transfer were characterized to establish the presence of markers other than lac+ on the episomes formed in Lac+ transductants of SC1402 Hfr13 Δpro-lac pur+. SC1500 Δ111 argF strR lacks both E. coli K12 genes encoding ornithine transcarbamylase activities; it is argF and lacks the argF gene which is included within the deletion X111. The F' lac containing exconjugants of SC1500 are Arg+. Thus the counter-clockwise “end” of the episomes is between argF and lac loci.

Other markers of the episomes were determined using two types of donors with consistent results. The F' type transductants were used as donors. Also Δ111 recA1/F lac exconjugants of SC0622 Δ111 recA1 (produced as described above) were used as donors. With recA1 strains as donors, mobilization of chromosomal markers by crossing over is insignificant. To score for the presence of proC+, purE+, and gal+ genes on the episomes, Lac+ StrR exconjugants of MC100 lacY proC purE galK strR were selected and replicated to suitable minimal media. To score for the presence of lon+ (cap+) on episomes, Lac+StrR exconjugants of MC102 lacZ capR9 strR were selected and subcloned. CapR9 strains exude capsu-
Markers of episomes formed in \textit{Lac}\textsuperscript{+} transductants of \textit{SC1402} Hfr13 \textit{\Delta}pro\textit{\A}\textit{lac} pur\textsuperscript{+}

<table>
<thead>
<tr>
<th>Transductant number</th>
<th>arg\textsuperscript{+}</th>
<th>lac\textsuperscript{+}</th>
<th>proC\textsuperscript{+}</th>
<th>lon\textsuperscript{+}</th>
<th>purE\textsuperscript{+}</th>
<th>gal\textsuperscript{+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>6, 9</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4, 12, 14</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5, 13, 16, 18</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The donor for transduction was \textit{\chi316 F-arg}+\textit{lac}+\textit{pro}+\textit{lon}+\textit{pur}+\textit{gal}+. For transductants 4, 5, 16 and 18, Hfr forms were detected during the purification.

lar polysaccharide which confers a mucoid appearance to their colonies at 37\textdegree, while their \textit{capR9/F' lac}+\textit{cap}+ derivatives are opaque and compact (Markovitz 1964). The episomes comprise a genetically overlapping family (Table 4). None carry \textit{galK}+. \textit{PurE}+ is carried on \textit{F'6} and \textit{F'9} episomes from transductants \textit{SC1416} and \textit{SC1419}, respectively.

\textit{PurE}+ genes of the \textit{F' lac}+\textit{proC}+\textit{lon}+\textit{purE}+ episomes are almost certainly derived from the recipient line: Some P1 virions might cotransport \textit{lac}+ and \textit{purE}+, as the P1 virion chromosome and the \textit{lac}+-\textit{lon}+-\textit{purE}+ chromosome segment are about the same length (Figure 1). But coinheritance of \textit{lac}+ and \textit{purE}+ during P1 transduction cannot be detected (Table 2). In fact there is only 0.1\% coinheritance of \textit{lon}+ with \textit{lac}+ during transduction (Markovitz 1964), and the \textit{lac}+–\textit{lon}+ segment is only about half the length of the \textit{lac}+–\textit{lon}+–\textit{purE}+ segment.

The genotype of \textit{SC1416}: If the host chromosome of \textit{SC1416} \textit{\Delta}pro\textit{\A}–\textit{lac} /\textit{F'6} retains \textit{purE}+, the \textit{purE}+ gene was in some way duplicated in the transductant line. A proposal for a genetically legitimate duplication process is developed in the Discussion. However, the following alternative must also be considered. There is only one \textit{purE}+ gene in the transductant, and the host Hfr13 \textit{\Delta}pro\textit{\A}–\textit{lac} \textit{purE}+ chromosome in some way suffered a \textit{purE} deletion during the formation of the \textit{F'6 lac}+ \textit{purE}+ episome.

To test the alternative, it suffices to determine the Pur character of \textit{F-} segregants of \textit{SC1416}. Each of ten independently isolated \textit{Lac}– segregants of \textit{SC1416} was found to be \textit{Pur}+. However, it must be demonstrated that such segregants do arise without crossing over with the episome. The qualification is essential. If the hypothetical \textit{purE}+ region deleted from the chromosome and transferred to the episome contains genes essential for viability, then only \textit{Lac}– \textit{Pur}+ segregants which had recovered \textit{purE}+ by crossing over prior to episome loss could be obtained. To block crossing over prior to \textit{F'6} loss a \textit{recA1} defect was introduced into \textit{SC1416} in two steps. First \textit{SC1426 mal}+ \textit{thyA/F'6} was selected as a spontaneous mutant of \textit{SC1416 mal}+ \textit{thy}+/\textit{F'6}. It was confirmed that \textit{SC1416} still yields \textit{Lac}– \textit{Pur}+ segregants. Then \textit{SC1416} was converted to an \textit{F-} phenocopy by growth at 25\textdegree. \textit{Thy}+–\textit{recA1} chromosome segments were introduced through a short conjugal cross with \textit{SC0701 thy}+ \textit{recA1 mal}+ (see Table 1). \textit{Mal}+ \textit{Thy}+
exconjugants were selected and Rec− Lac+ types were purified. These exconjugants were the desired Rec− derivatives of the original SC1416 ΔproA−lac rec+ strain.

For the SC1427 ΔproA−lac recAI trp+/F'6 derivative, it is shown that there is little conjugal mobilization of the host trp+ gene (Table 3). This lack of chromosomal mobilization confirms that there is little crossing over between episome and chromosome occurring in the Rec− background. For each of ten distinct Rec− exconjugants of SC1416 ΔproA−lac/F'6, ten Lac− segregants were isolated. All Lac− segregants were Pur+. Thus it can be concluded that the ΔproA−lac recAI chromosomes do encode purE+.

It is conceivable that there was a purE+ deletion in the SC1416 ΔproA−lac/F'6 transductant line, which was in some way eliminated before the introduction of the recAI alleles. However the simplest interpretation of the results is that the host chromosome of the SC1416 transductant line never lost its purE+ gene.

Other transductants: Among the lac+ transductants which are not efficient and early lac+ gene donors, three types have been distinguished. Two transductants transfer lac+ by conjugation after 90 minutes. I presume that lacV is integrated between F and ΔproA−lac loci, and that lacV does not genetically overlap the F locus. It appears that one transductant is physiologically unfit and/or that the heterozygous genotype is exceedingly unstable. Even colonies grown on minimal lactose proline agar (on which proline may be catabolized as a carbon source) contain more than 90% Lac− segregants. This transductant has been set aside for a separate characterization.

The remaining six transductants are all more unstable than the transductants rapidly segregating F' strains. While F− segregants are readily obtained by picking Lac− sectors of the F' strains, F− segregants have not been found among the Lac− segregants of these six transductants. In crosses with SC1500 Δ111 strR, F'lac+-carrying exconjugants are formed, but with only 1–10% the efficiency of the crosses with ΔproA−lac/F'lac strains. The longest episome so isolated carries lac+ and proC+.

A tentative explanation of these characteristics is developed in the DISCUSSION.

DISCUSSION

Heterozygote lines produced through lac+ transduction of an Hfr13 ΔproA−lac recipient have been analyzed in this study. Some transductants segregate F' strains. It is plausible that the segregation event is a legitimate crossover within a recombinant Hfr13 ΔproA−lac/lacV chromosome, which yields companion F'lac and F− ΔproA−lac chromosomal products (Figure 2). The purE+ gene is not contransduced with lac+. But two of the ten F' strains carry purE+ on their episomes (Table 4). Evidence that purE+ is also encoded in the host chromosome has been sought and obtained for one of these exceptional heterozygotes. Hence the duplicated purE+ genes of the transductant both appear to be derived from the recipient line. This is the most intriguing observation of this study. How did the recipient gene duplication occur?

A reasonable possibility is that the partial chromosomal replication accompanied an initial crossing-over step in fragment integration. A structure suitable
Figure 3.—A model for purE gene duplication during lac+ fragment integration. A single crossover is initiated (i) by an attack of the lac+ (L) fragment strand with a 3' terminus at a homologous chromosomal locus to the right of the Δpro-A-lac deletion (Δ) and the F locus (F). Ligation of the fragment strand with a recipient chromosomal strand completes the formation of a fork, from which some type of DNA replication (r) proceeds. Unknown factors control the extent of the replicative process which proceeds through purE (P) for the case depicted. A breakage of the previously continuous recipient chromosomal strand terminates (t) the replication step. Chromosome continuity is established by fusion (f), with the fusion joint (jnt) depicted as a dotted line.

for the initiation of some type of chromosomal replication could be formed as a strand of a transduced fragment requires continuity with a homologous recipient chromosomal strand (Figure 3). If replication proceeds, the fragment branch of the fork is genetically elongated. Thus when integration is completed by replication fork breakage and a fusion of chromosome ends, the resulting duplication can include recipient genes which were not represented on the initially absorbed fragment. The elongation step could proceed whether a 3' or 5' strand of the fragment acquires continuity with the chromosome initially. However, I have argued that fragment ends generally acquire single-stranded 3' termini in recombination-proficient bacteria, and that the priming of synthesis from a 3' terminus on a heterologous template strand is the critical stabilizing step in some fusion events (Stodolsky 1973a). A single-stranded 3' terminus is specified in Figure 3 only to maintain compatibility with these ideas.

The length of the proposed replication step cannot be estimated from data of this report for at least three reasons. The portion of the duplication derived directly from the absorbed lac+ fragments is not known. Some duplicated genes may be degraded prior to the establishment of the fusion joint. The rate of degradation of the lac+ fragment end remaining exposed during replication is not known; if DNA end degradation is rapid, then the selection for transductants...
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rctaining lac+ would also constitute a selection for recombinants formed through shorter replication steps. A minimal estimate of the length of the longest replication step detected can be made. There is only about 0.1% coinheritance of lac and lon during transduction (MARKOVITZ 1964). It is reasonable to assume, therefore, that the lon+-purE+ segments of the two ΔproC+-lac+lon+ purE+/F'lon+ proc+ lon+ purE+ transductants are derived from the recipient chromosome. The lon+-purE+ chromosome segment is about 18 microns long.

Six of the nineteen transductants analyzed are unusual in that their Lac- segregants are predominantly or exclusively like the Hfr13 ΔproC+-lac recipient for transduction, and yet they are donors of F' episomes. As a tentative explanation of these characteristics, I suggest that the transductants are double males with F' episomes integrated into the host chromosomes. For instance, a strain with ΔproC+--F--proc+--int--lac+--F--proc+--lon+ gene linkages would correspond to a Hfr13 ΔproC+-lac proc+ lon+ host with an integrated F'proc+ procF episome. Its Lac- segregants could only be Hfrs. During conjugation the proximal segment transferred would sometimes include transfer origin--proc+--int--lac+--F--proc+--lon. A segment of this type could give rise to a circular F'proc+ procF episome in the recipient through intramolecular crossing over.

There is a trivial course of events through which a double male transductant could be formed. In a binucleate Hfr13 ΔproC+-lac recipient, all steps in lac+ fragment integration and F' excision (Figure 2) could proceed in a single generation. The cell then contains the F'proc, its companion recombinant proc--F-lac+ chromosome, and the Hfr13 ΔproC+-lac chromosome which did not participate in the recombinant formation. Thus upon cell division the F'proc genote could segregate with the Hfr13 chromosome. Though the F'proc genote could not replicate autonomously in the Hfr host, it could be integrated into the host ΔproC+--F--procF chromosome, yielding a double male recombinant. A more exotic mode of origin would be the following. During lac+ fragment integration, the replicative step in crossing over is initiated between ΔproC+-lac and F loci. The replication traverses the F factor, and the duplicated F genes are conserved during a subsequent chromosome breakage and fusion. In this case the recombinant product is a double male.

With the selection system utilized in this study only heterozygous Lac+ transductants can be obtained. But heterozygotes are also formed with donor and recipient are essentially isogenic and without barriers to the formation of haploid recombinants. In the initial analysis of transduction mediated by P1, it was observed that transductant colonies were frequently a mixture of haploids (LENNOX 1955). It now seems plausible that the genetically diverse haploids were segregants of an ancestral heterozygous recombinant. Using a transduction system for which persisting heterozygotes within a clone are readily detected, MARKOVITZ (1964) found that about 30% of the transductants for the procC-lon region are heterozygotes. However the frequency of heterozygote formation during transduction is not uniform around the E. coli chromosome. Only about 0.4% of the transductants for sup-159 have tandem duplications (HILL et al.
FIGURE 4.—Dependence of the outcome of integration processes on the mode of replicative step termination. The integration of a fragment (A) into a homologous chromosomal region is initiated as in Figure 3 and replication (r) proceeds. Replication step terminations, in which the branch of a fork continuous with the fragment DNA are broken and conserved, are respectively designated (t') and (t). Temporal simultaneity of termination steps is not assumed. When replication step terminations (t' and t) culminate in chromosome breakage, chromosome continuity can be restored by crossing over (c) or fusion (f).

1969). What factors control the ratio of haploid to heterozygous transductant formation?

Perhaps a controlling factor is the mode of replication step termination in crossing over. Consider an intermediate for fragment integration (Figure 4) formed through two crossovers initiated as depicted in Figure 3. There are three ways in which the intermediate can be disrupted. If the forks continuous with the fragment are both broken, the fragment integration is aborted. If both forks continuous with the corresponding recipient chromosomal segment are broken, the recombinant is haploid. But if alternative forks are broken, the immediate product has genetically overlapping ends (or a terminal repetition since the *E. coli* chromosome is circular). A joining of the ends by legitimate crossing over will again result in the appearance of a haploid recombinant. But as previously noted (Stodolsky, et al. 1972) this mode of end joining is competitive with joining through fusion, and the latter process leads to the formation of a tandem duplication. Thus in the context of this scheme, a genetically local control of the mode of replicative fork disruption can result in diverse frequencies for the formation of terminally repetitious recombinant intermediates for different chromosomal regions; and the formation of such intermediates for different chromosomal regions; and the formation of such intermediates may culminate in tandem duplication formation. The striking effects of local gene expression on fragment integration described by Drexler (1972) do show that some steps in integration are susceptible to locus-specific control and variation.

The most decisive evidence for the occurrence of recombination-associated replication has been obtained by Stahl and coworkers (1971, 1973) in analyses of crosses between density-labeled bacteriophage lambda. The occurrence of such replication provides a basis for explaining certain gene conversion phenomena (Boon and Zinder 1971; Stadler and Towe 1971). The evidence presented herein is novel in character; the participants in crossing over are recovered as
part of a single recombinant structure, the Δpreα-lac/lacV chromosome, rather than as separated recombinant products.

A duplication phenomenon which may be related to that described herein has been analyzed by Hill, Schiffer and Berg (1969). They observed that tandem duplications are constructed with high efficiency in a haploid recipient when the transduced fragment encodes both the selected donor marker and the joint of the donor’s duplication. This phenomenon was explained in terms of sequences of legitimate crossovers involving the fragment and two sister recipient chromosomes, through which a segment of one sister chromosome is deleted and utilized in the reconstruction of the duplication. An alternative explanation for this reconstruction phenomenon is as follows. The genes of the duplication which were not cotransduced with the repeat point were added to the fragment in the recipient, through a replication accompanying crossing over. The unit repeat can then be added to the recipient chromosome through purely legitimate crossovers and a companion deletion needn’t be produced. Boon and Zinder (1971) have also commented upon this possibility.

The trick utilized herein to produce the F’episomes in Hfr recipients can undoubtedly be extended to Hfr strains for which F is not adjacent to a deletion. Perform an a−b+ x a+-F−b− cross, selecting for transductants with an A+B+ phenotype. If a and b are very tightly linked genes in the donor, the contribution of a+ b+ haploid recombinants to the transductant population will be minimal, and an enrichment for partial diploids will therefore be achieved. Among the heterozygous recombinants, those with a−b−-jnt-a+-F−b− chromosomes will segregate a-b-/F’a+b+ strains. The technique of heterozygote selection has sufficed for a related task—the isolation of transductants with specialized transducing derivatives of P1 as a+b− (P1da b+) lysogens (Stodolsky 1973b). This study and its predecessor (Stodolsky, Rae and Mullenbach 1972) were in fact initiated as control experiments without P1 helper, in a study of the origins of specialized transducing derivatives of bacteriophage P1 (Rae and Stodolsky 1974).

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LITERATURE CITED


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