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Selection of *lac* Gene Fusions In Vivo: *ompR-lacZ* Fusions that Define a Functional Domain of the *ompR* Gene Product

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We describe a simple method for selecting *Escherichia coli* mutants that carry gene fusions between a cloned gene and *lacZ*. We test this technique with the *ompR* gene, which codes for a positive regulatory factor in porin synthesis. A number of OmpR-LacZ hybrid proteins are examined, and several unusual phenotypes associated with these protein fusions are described. Evidence is presented to support the two-domain model for *ompR* proposed previously (Hall and Silhavy, J. Mol. Biol. 151:1–15). In addition, one of the *ompR-lacZ* fusions exhibits a dominant OmpR$^-$ phenotype. The utility of isolating a series of *lacZ* gene fusions to any target gene is discussed.

Studies that use *lac* gene fusions as an analytical technique have increased dramatically in recent years because of the development of rapid and simple methods for the isolation of gene fusions in vivo (5, 7) and the construction of gene fusions in vitro (6, 9). Certain biological problems can be best studied by constructing hybrid genes that code for hybrid proteins. For example, *lacZ* gene fusions have been extensively used to isolate and characterize chimeric proteins (10). Previous studies have shown that the replacement of up to the first 26 amino acids of the LacZ monomer with various numbers of unrelated residues does not affect β-galactosidase activity (31). Such two-part proteins can be isolated that retain certain functions of the non-LacZ residues (10, 11, 22), and unique properties attributed to these bifunctional constructs can, in conjunction with Lac phenotypes, be exploited for genetic analysis (10).

The construction of *lacZ* protein fusions in general involves the activation of a genetically inactivated *lacZ* gene. The original in vivo methods used a structural gene mutation at codon 17 of *lacZ* to select for deletion events that resulted in the replacement of at least the first 17 residues of LacZ (5, 22). A further refinement utilized a *lacZ* segment truncated at codon 8, which is nonfunctional, and incorporated into phage transposons (7; E. Bremer, personal communication). Upon transposition, insertions in frame with an actively transcribed and translated gene result in the formation of a LacZ$^+$ protein fusion. Finally, in vitro techniques have been described that employ a series of cloning vectors carrying the *lacZ* gene truncated at either codon 8 or codon 5 (9). In these vectors there are several unique restriction enzyme sites immediately adjacent to *lacZ* that serve as cloning sites for exogenous DNA fragments. The insertion of any DNA sequence that provides a translation start site in frame with *lacZ* can activate these vectors to express *lacZ*. Transcription of the *lacZ* region on these vectors can be provided by promoter signals encoded on the inserted DNA fragment or carried in a different region of the vector (13, 25, 28).

One existing limitation of the vectors for the in vitro construction of *lacZ* fusions is the necessity to form a gene fusion by directed DNA insertion. This requires either the presence of specific restriction enzyme sites within the target gene or the use of nuclease procedures to generate random fragment ends, e.g., Bal 31 treatment (12). In practice, the ability to isolate many protein fusions to any target gene is limited by the lack of either specific enzyme mapping data for the target gene or the inefficiency of bimolecular cloning reactions with sheared or Bal 31-treated DNAs. To overcome these limitations, we have devised a simple selection technique which can be used in conjunction with some existing *lacZ* fusion vectors to yield a wide range of essentially random protein fusions to any target DNA.

We have chosen to demonstrate the utility of our new methodology by creating a series of *ompR-lacZ* protein fusions. The *ompR* gene of *Escherichia coli* codes for a positive regulator of porin gene expression (14–16, 24). The *ompR* structural gene has been cloned previously (26), and the DNA sequence of this region has been published (30). This information provided us with an opportunity to test our new approach as well as to examine the behavior of various OmpR-LacZ chimeric proteins.

The essential feature of our selective system is that it is very easy to isolate an insertion of a DNA fragment into a *lacZ* cloning vector adjacent to the truncated *lacZ* gene that does not form a functional (LacZ$^+$) fusion. In practice, the majority of DNA fragments inserted into these vectors do not form a functional hybrid *lacZ* gene. Using such a LacZ$^-$ construction, we can apply a selection for LacZ$^+$ clones that can be shown to result from independent, essentially random, deletion events fusing the target gene sequences to *lacZ*.

MATERIALS AND METHODS

Strains. The *E. coli* strains used in these experiments are listed in Table 1. The selection of Lac$^+$ gene fusions required a Δ*lac* strain that had been lysogenized with λp1048 (see below). The lysogen used in this study was strain MBM7060, although similar derivatives of other Δ*lac* laboratory strains have also been constructed and used in this system. These include MC4100 (5), MC1000 (8), and MH3000 (28).

Media and reagents. Media, culture conditions, DNA transformation protocols, and conditions of sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been previously described (25). Lactose MacConkey medium (Difco Laboratories) contained 1% lactose. Ampicillin was added to all media at a concentration of 150 μg/ml. Assay of β-galactosidase activity was by the method of Miller (19). *Eco*RI oligonucleotide linker (5′ CCGAATTCGG 3′) was purchased from Collaborative Research, Inc.

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TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Relevant genotype	Source or reference
Strains		
MBM7014	F^- *araC*(Am) *araD* Δ(*argF-lac*)*U169 trp*(Am) *malB*(Am) *rpsL relA thi*	2
MBM7060	MBM7014(λp1048)	This study
MC4100	F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25*	5
MH1160	MC4100 *ompR101*	15
MC1000	F− *araD139* Δ(*araAB01C-leu*) *7679 galUK* Δ*lacX74 rpsL thi*	8
Bacteriophages		
λp1041	Φ(*tyrT'-lacZ*$^+$ *Y*$^+$)*1041*	This study
λp1048	Φ(*tyrT'-lacY*$^+$)*1048*	This study
λNF1955	*'lacZ lacY*$^+$	17
Plasmids		
pMLB1034	*'lacZY'*	29
pMLB1010	Φ(*'trp-lacZ*$^+$)*W205*	27
pMLB1041	Φ(*tyrT'-lacZ*$^+$ *Y'*)	27
pMLB1048	Φ(*tyrT'-'lacZY'*)	27

Construction of λp1048. The transducing phage λp1048 is an *att*$^+$ phage that can be used to provide any cell with constitutive synthesis of lactose permease. The construction of this phage proceeded through a series of in vitro and in vivo steps. We utilized two previously described plasmids in construction of the λp1048. Plasmid pMLB1041 carries a *tyrT-lacZ* operon fusion constructed in vitro (27). Plasmid pMLB1048, a derivative of pMLB1041, carries a deletion of DNA sequences from nucleotide +57 of the *tyrT* structural gene to nucleotide +2796 of the *lacZ* structural gene (27). We constructed a specialized λ transducing phage carrying the entire *tyrT-lacZ* fusion from plasmid pMBL1041, using the vector λNF1955 (17). The resulting phage, λp1041, was propagated on a strain carrying plasmid pMLB1048 to obtain a transducing phage with the same deletion as this plasmid. These recombinants occurred at a frequency of 0.5 to 1%, as assayed by the number of colorless plaques on 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) medium. Finally, the *c*I857 and *S*am100 alleles originally carried by λNF1955 were replaced by the corresponding wild-type alleles by an in vivo cross with wild-type λ. The resulting phage λp1048 (Fig. 1), which carried only the terminal 91 codons of *lacZ*, transduced a constitutively expressed *tyrT-lacY*$^+$ operon fusion. Lysogens of this *att*$^+$ phage were isolated by streaking from the turbid center of phage plaques or spots.

Construction of pMLB952. DNA manipulations were performed according to previously published protocols (25). The construction of pMLB952 required subcloning of the *ompR* gene from plasmid pRT516, a derivative of pRT506 (26). According to previously published data, there is a naturally occurring *Eco*RI site after the *ompR* gene within the *envZ* structural gene (26, 30). To aid in the subcloning of *ompR*, we placed a synthetic *Eco*RI oligonucleotide linker at the unique *Sma*I site in bacterial DNA preceding the *ompR* structural gene on pRT516. This allowed us to isolate a 1,288-base-pair (bp) *Eco*RI fragment carrying the intact *ompR* gene and a portion of the beginning of the *lacZ* gene.

This fragment was subcloned into the *Eco*RI site of plasmid pMLB1034 (29). This vector is a derivative of plasmid pBR322 (3), carrying a DNA fragment from codon 9 of *lacZ* to the *Ava*I site at codon 70 of *lacY* (4). The *lac* DNA replaces the *tet* region from the *Eco*RI site at position 4360 to the *Ava*I site at position 1425 on the pBR322 map (23). The *lacZ* sequence was oriented in a clockwise fashion. Cells harboring this plasmid were phenotypically $LacZ^-$ and $LacY^-$. Activation of *lacZ* expression required the insertion of at least a functional ribosome-binding site into the reading frame of *lacZ*.

Isolates of an *ompR101* strain harboring these plasmids carrying the *ompR* fragment in either orientation at the *Eco*RI site of pMLB1034 were phenotypically $OmpR^+$. We interpret this result as showing that the *ompR* promoter was included in this 1,288-bp fragment. We selected one clone, pMLB952, that carried the *ompR* gene in the same orientation as *lacZ* (Fig. 2).

Analysis of *ompR* expression. The expression of both the *ompF* and *ompC* genes is regulated by two genes, *ompR* and *envZ* (16). Mutations mapping in the *ompR* gene abolish expression of one or both of the porin genes. It is simple to detect the presence of these gene products since both are receptor proteins for different bacteriophages. OmpF is the receptor for phage K20, and OmpC is the receptor for phage hy20. The function of *ompR* can be tested by scoring for sensitivity to these two phages (14).

RESULTS

Experimental design. These experiments use the *lacZ* cloning vector pMLB1034 (see above) (29). Rather than directly cloning a DNA fragment that would, upon insertion, activate *lacZ* expression from pMLB1034, we wanted to test for the occurrence of spontaneous DNA rearrangements that result in the activation of *lacZ* in a pMLB1034 clone carrying a target gene not fused to *lacZ*.

The starting vector pMLB1034 carries only a portion of *lacY*, and therefore we had to provide an intact copy of *lacY* to select for utilization of lactose. This was accomplished by the construction of a *tryT-lacY* operon fusion carried by phage λp1048 (Fig. 1). The *tyrT* gene was chosen because this stable RNA gene possesses a strong constitutive promoter (2). Strain MBM7060, which is a lysogen of λp1048, is

FIG. 1. Structure of λp1048. This map represents the structure of the λ transducing phage λp1048. Various phage genes are indicated. This phage, which is derived from the cloning vector λNF1955, is essentially wild-type λ with a *tyrT-lacY* operon fusion inserted in the *b2* region. Sizes are given in kilobases, and the bacterial insert is enlarged. The vertical arrows indicate *Eco*RI sites, and the coordinates (−248 and +58) show the extent of the *tyrT* gene material (dotted box). A portion of *lacZ* (solid box) and the structural gene for *lacY* (striped box) are drawn to scale. A prime indicates that part of the labeled gene is missing in the direction indicated. The position of other sites (see text) corresponds to the nucleotide numbers within the specified structural genes: *tyrT* (27), *lacZ* (18), and *lacY* (4). The horizontal arrow shows the origin and direction of transcription.

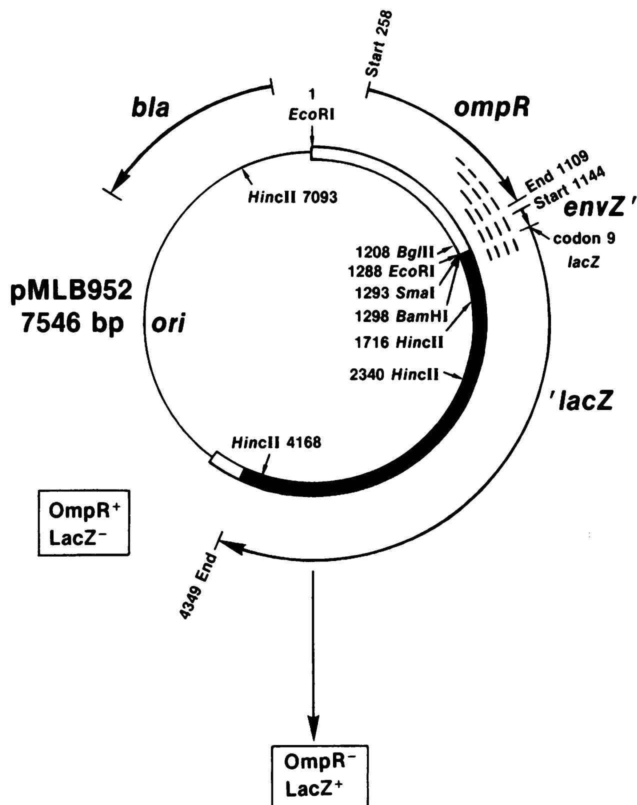

FIG. 2. Map of plasmid pMLB952. The thin line represents pBR322 material. The boxed regions represent bacterial sequences from *ompR*, *envZ*, *lacZ*, and part of *lacY* (not labeled). The *lacZ* structural gene is the solid box. The first base of the *Eco*RI site is taken as coordinate 1, and the locations of the first base of restriction sites, the first base of the first codon of bacterial genes, and the last base of the last codons of these genes are labeled accordingly. The origin of replication, *ori*, of pBR322 is labeled, as well as the structural gene for the β-lactamase (*bla*). The arrows indicate the orientation of the different structural genes. The dashed arcs illustrate the extent of various deletions resulting in *OmpR-lacZ* gene fusions.

phenotypically LacY$^+$ and was used in the selections described here.

The target gene in our initial experiments was the *ompR* gene. A DNA fragment carrying the entire *ompR* gene was cloned into the *Eco*RI site adjacent to the truncated *lacZ* gene in pMLB1034 (see above). The resulting plasmid, in which the *ompR* structural gene was oriented in the same direction as *lacZ*, did not express *lacZ* because there was no translation start site in frame with the structural gene. However, this plasmid, pMLB952 (Fig. 2), did express *ompR* because the entire gene was intact. The strain MBM7060(pMLB952) produced no detectable β-galactosidase activity.

Selection of Lac$^+$ derivatives of MBM7060(pMLB952). Two selections for Lac$^+$ clones (requiring both *lacZ* and *lacY* expression) were performed. In the first method, five different colonies were inoculated into 5 ml each of LB broth containing 125 μg of ampicillin per ml. These cultures were grown at 37°C overnight, and the cells were collected by centrifugation at 3,000 × *g*. The cell pellets were suspended in 0.3 ml of M63 medium, and each sample was spread on a single M63 minimal plate containing lactose (0.2%) and ampicillin (125 μg/ml). These selective plates were incubated for 2 to 3 days at 37°C. The average number of spontaneous Lac$^+$ mutants in five overnight cultures was estimated as 10^{-10} by this direct selection on minimal plates containing lactose.

The second method takes advantage of the ability of Lac$^+$ clones to grow out of a Lac$^-$ lawn on lactose indicator plates. This phenomenon is due to the ability of the Lac$^+$ mutants to utilize the high levels (1%) of lactose in lactose MacConkey medium. When a lactose MacConkey plate spread or streaked with the Lac$^-$ parent, MBM7060(pMLB952), was incubated at 37°C for 4 to 5 days, Lac$^+$ papillae began to emerge from areas of confluent growth as well as within individual Lac$^-$ colonies. When incubation was continued, almost every individual colony eventually gave rise to a Lac$^+$ papilla. We observed a relatively low number of Lac$^+$ clones in individual overnight cultures with the first method. This contrasts with the relatively large number of Lac$^+$ papillae that arose on a single lactose MacConkey plate. Since these Lac$^+$ papillae were physically separate and clearly arose as the Lac$^+$ lawn was growing, we consider each of these papillae the result of an independent DNA rearrangement. This selection procedure permits the isolation of hundreds of independent Lac$^+$ mutants from a single lactose MacConkey plate.

We also tested the requirements for *LacY* expression in our selection method. We transformed plasmid pMB952 into strain MBM7014 (LacY$^-$) and compared the number of spontaneous Lac$^+$ papillae that occurred on MacConkey medium with the number of papillae that arose from the same plasmid in strain MBM7060 (LacY$^+$). Once again, after 5 to 7 days at 37°C, MBM7060(pMLB952) showed hundreds of Lac$^+$ papillae. However, no papillae were observed with MBM7014(pMLB952). We do not know what level of *lacZ* expression is required in the absence of *lacY* expression to obtain outgrowth of Lac$^+$ papillae on a lactose MacConkey plate.

Isolation and analysis of plasmid DNA from Lac$^+$ mutants of MBM7060(pMLB952). We isolated plasmid DNA from 40 spontaneous Lac$^+$ mutants selected on lactose MacConkey medium. We analyzed the plasmid DNAs by restriction enzyme digestion to determine what type of DNA rearrangement had activated *lacZ* expression. From the predicted map of pMLB952 (Fig. 2), we chose the enzyme *Hin*cII for analysis. There are two *Hin*cII sites defining a 2,169-bp fragment that extends from the *bla* gene to codon 146 of *lacZ*, within which we expected to find any physical alterations. The remaining *Hin*cII fragments from the pMLB952 plasmid originate from the *lac* sequences or from pBR322 sequences and should remain unchanged in our mutants. In addition to detecting alterations to the 2,169-bp *Hin*cII fragment, we expected to observe a mixture of parent and mutant plasmids from any single Lac$^+$ clone. This is due in part to the high copy number of pBR322 derivative plasmids. Since LacZ$^+$ is dominant to LacZ$^-$, a single mutant LacZ$^+$ plasmid will confer a Lac$^+$ phenotype in our selection. All of the clones analyzed did indeed carry both parent plasmids (indicated by the presence of the 2,169-bp *Hin*cII fragment) as well as various deletion derivatives that yielded unique *Hin*cII fragments smaller than 2,169 bp (data not shown).

Based upon the screening with *Hin*cII, we were able to group the 40 original Lac$^+$ clones into 10 classes according to the relative size of the apparent DNA deletion. A representative from each size class was chosen for further analysis. By repeated retransformation, or simply by restreaking the original clones, we were able to segregate a progeny strain that harbored only the deletion (*lacZ*$^+$) plasmid. The

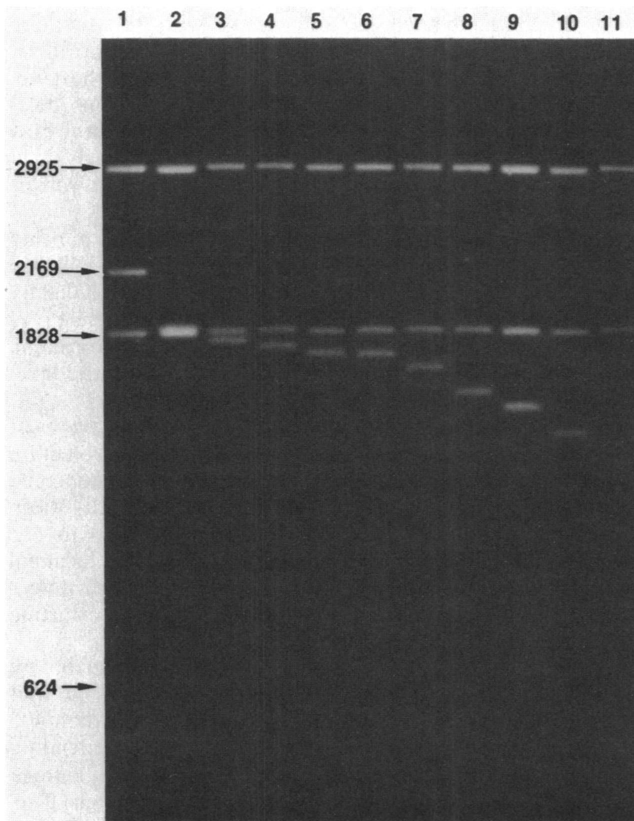

FIG. 3. Agarose gel electrophoresis of plasmid DNAs from the Lac$^+$ clones. This is a 1.7% agarose gel display of *Hin*cII digests of ~1 μg of plasmid DNA. The lanes correspond to the following plasmids: lane 1 (LacZ$^-$ control), pMLB952; lane 2, pMLB954; lane 3, pMLB955; lane 4, pMLB956; lane 5, pMLB957; lane 6, pMLB958; lane 7, pMLB959; lane 8, pMLB960; lane 9, pMLB961; lane 10, pMLB962; lane 11, pMLB963. The sizes (in bp) of the *Hin*cII fragments from pMLB952 are indicated. The locations of these DNA fragments can be found by referring to Fig. 2.

results of *Hin*cII digestion of plasmid DNA from these representative clones is shown in Fig. 3. The smallest deletion (~319 bp) is of sufficient size to enter the *ompR* structural gene, whereas the largest deletion (~ 916 bp) will remain with *ompR*. Therefore, we can tentatively conclude that all of the Lac$^+$ clones harbor *ompR-lacZ* protein fusions.

In addition to analyzing the size of the DNA deletions, we also tested each plasmid for the presence of the original DNA linker sites adjacent to *lacZ*. The synthetic DNA including these sites encodes a number of sense codons in frame with *lacZ* (29). We examined the plasmids from our Lac$^+$ clones for retention of the *Eco*RI, *Sma*I, and *Bam*HI sites adjacent to *lacZ*. The results of this analysis with a summary of the deletion sizes are presented in Fig. 4.

Analysis of the *ompR-lacZ* hybrid proteins encoded by the LacZ$^+$ plasmids. Our restriction enzyme analysis of the individual plasmids revealed a progression of deletions within the *ompR* structural gene. Because these plasmids expressed *lacZ*, the deletion endpoints within the *lacZ* structural gene must be confined to the region between the first nonsense codon in frame with *lacZ* and codon 26 of *lacZ*. This means that one deletion endpoint must be confined to a relatively small stretch of DNA (see below). Essentially, the deletion endpoint within *lacZ* sequences was fixed, whereas

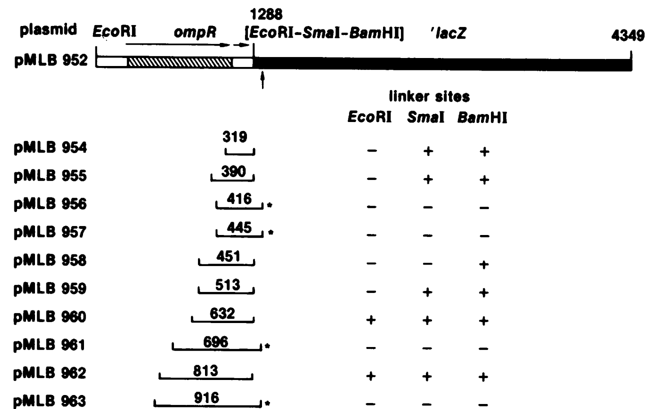

FIG. 4. Summary of DNA analysis of LacZ$^+$ clones. A portion of the map of pMLB952 is shown on the first line. The structural gene for *ompR* (striped box) and *lacZ* (solid box) is shown. The vertical arrow indicates the furthest deletion of *lacZ* material that is consistent with a LacZ$^+$ phenotype (taken here as codon 26; see text). The brackets represent the location and size (in bp) of deletion events as analyzed at the DNA level (Fig. 3). DNA fragment size was determined with a BRL model NA2 digitizer. The average error for this measurement was 0.5%. The presence or absence of the three linker sites is also shown. An * at the right end of the brackets indicates that this deletion endpoint lies between the linker site and codon 26 of *lacZ*.

the endpoint within *ompR* sequences was variable. Therefore, we expected that the larger the deletion, the smaller the hybrid protein specified by that gene fusion. We analyzed the hybrid proteins from the 10 clones by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 5) and

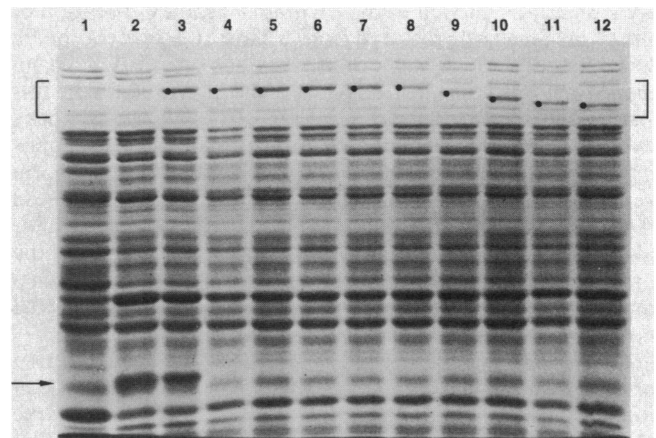

FIG. 5. Polyacrylamide gel electrophoresis of proteins from various strains. Strains were grown, extracted, and harvested as previously described (25). Protein extracts were electrophoresed in an 8% sodium dodecyl sulfate-polyacrylamide gel and stained with 0.2% Coomassie blue. The lanes contain extracts from the following strains: lane 1, MH1160 (no plasmid); lane 2, MH1160(pMLB952); lane 3, HM1160(pMLB954); lane 4, MH1160(pMLB955); lane 5, MH1160(pMLB956); lane 6, MH1160(pMLB957); lane 7, MH1160(pMLB958); lane 8, MH1160(pMLB959); lane 9, MH1160(pMLB960); lane 10, MH1160(pMLB961); lane 11, MH1160(pMLB962); lane 12, MH1160(pMLB963). The vertical bracket indicates the region of the gel in which the hybrid proteins are found. The positions of various OmpR-LacZ hybrid monomers are indicated by dots. The horizontal arrow indicates the position of the OmpF and OmpC porin bands. These two gene products are not resolved in this gel system.

TABLE 2. Sensitivity of different clones to phages hy2 and K20 as measurement of *ompC* and *ompF* expression

Plasmid	OmpR residues[a] (% wild type)	MH1160 (OmpR⁻)[b]		MC4100 (OmpR⁺)[b]	
		hy2 (OmpC)	K20 (OmpF)	hy2 (OmpC)	K20 (OmpF)
None		R	R	S	S
pMLB952	284 (100)	S	S	S	S
pMLB954	238 (84)	R	S	S	S
pMLB955	219 (77)	R	R	S	S
pMLB956	214 (75)	R	R	S	S
pMLB957	209 (74)	R	R	S	S
pMLB958	207 (73)	R	R	S	S
pMLB959	173 (61)	R	R	S	S
pMLB960	134 (47)	R	R	R	R
pMLB961	125 (44)	R	R	S	S
pMLB962	73 (26)	R	R	S	S
pMLB963	52 (18)	R	R	S	S

[a] Contributions of residues from OmpR to the OmpR-LacZ hybrids were estimated from the size of the DNA deletions and the apparent molecular weight on sodium dodecyl sulfate-polyacrylamide gels.

[b] S, Sensitive; R, resistant.

found the expected relationship between deletion size and protein size, indicating that our clones did indeed carry *ompR-lacZ* hybrid genes that specified hybrid proteins.

OmpR phenotype of the *ompR-lacZ* fusions. The analysis of the proteins from our clones revealed an unexpected result. The strain in which this experiment was performed (MH1160) carries the chromosomal mutation *ompR101*. This allele inactivated the chromosomal *ompR* gene and abolished expression of the porin genes (Fig. 5, lane 1). As expected, the starting plasmid pMLB952, which did not express *lacZ*, was genotypically *ompR*$^+$; therefore, when pMLB952 was carried in the *ompR101* strain, expression of the porin genes was restored (Fig. 5, lane 2). However, much to our surprise one of the plasmids that encoded a hybrid *ompR-lacZ* protein also expressed the porin genes in this strain (Fig. 5, lane 3). To determine whether these clones expressed *ompR* function, we transformed these plasmids into various test strains and analyzed expression of the porin genes by testing for phage sensitivity (see above). These results (Table 2) confirmed that in the *ompR101* background, plasmid pMLB954 did indeed restore expression of the *ompF* gene. In addition, a second *ompR-lacZ* fusion plasmid, pMLB960, conferred an OmpR$^-$ phenotype to the normally wild-type (OmpR$^+$) strain MC4100 (Table 2).

LacZ expression from *ompR-lacZ* fusions. The construction of the different strains for examining the OmpR phenotype of the various plasmids allowed us to test the effect of the chromosomal *ompR* alleles upon expression of the plasmid-borne *ompR-lacZ* fusions. The results of β-galactosidase assays are presented in Table 3.

DISCUSSION

We set out to devise a technique that allows the selection of many LacZ$^+$ gene fusions to any target gene cloned on a high-copy-number plasmid. The synthesis of two gene products, β-galactosidase (*lacZ*) and lactose permease (*lacY*), are required for utilization of lactose. Similar in concept to the original selections devised by Miller et al. (20) and Mitchell et al. (21), our fusion events must generate a phenotypically LacZ$^+$ LacY$^+$ clone. We have tested this selection with several target genes. In total, several hundred spontaneous LacZ$^+$ protein fusions have been examined at the level of the plasmid DNA. Almost all of the events leading to a LacZ$^+$ fusion have been deletion events, with the exception of one clone in which a DNA insertion had occurred (J. Shultz, unpublished data). Although a role for short sequence homologies in the formation of spontaneous deletions has been proposed previously (1), we do not know how specific DNA sequences influence our selection. Analysis of the DNA sequence of various gene fusions selected in this system is in progress and will address this question.

The levels of *lacZ* expression from a pBR322-type plasmid required to obtain a Lac$^+$ phenotype in strain MBM7060 vary over a wide range. We have tested strain MBM7060 by transformation with plasmids that code for a range of β-galactosidase levels. In all cases, the resulting transformants were fully Lac$^+$ on minimal or indicator medium if the level of *lacZ* expression was above 100 U.

In addition to MBM7060, we have constructed lysogens of other common Δ*lac* strains and tested these in our selection system. Strain MBM7060 is Δ*lacU169* on the chromosome and carries phage λp1048 at the λ *att* site. All strain backgrounds tested worked as well as MBM7060 in our initial tests. Since the phage λp1048 carries only the terminal 91 codons of *lacZ*, there are no apparent genetic rearrangements that will affect the LacZ phenotype of our starting plasmids.

The selection of Lac$^+$ clones on lactose MacConkey media requires *lacY* expression. Results from our lab and other studies (13) have shown that a level of more than 400 units of β-galactosidase in a LacY$^-$ strain will, depending upon strain background, give a Lac$^+$ phenotype on lactose MacConkey indicator medium. However, although it is possible to distinguish LacY$^-$ cells harboring an *ompR-lacZ* fusion with lactose MacConkey medium, the formation of papillae is dependent upon *lacY* expression.

The relationship between deletion size and the sizes of the corresponding OmpR-LacZ hybrid proteins shows the expected correlation. Examination of the predicted DNA sequence of pMLB952 revealed the location of the first nonsense codon in the *lacZ* frame (Fig. 6). The first stop codon in frame with *lacZ* was a UGA codon that was encoded by the first two bases of the *Eco*RI site and the first base immediately preceding this site. Since protein fusions must form a DNA fusion in the *lacZ* frame, but not including this UGA codon, we expect to replace at least this codon in our LacZ$^+$ fusion clones. The *lacZ* endpoints of gene fusions

TABLE 3. Effect of chromosomal *ompR* alleles on expression of plasmid-borne *ompR-lacZ* fusions

Plasmid	β-galactosidase activity[a]		Ratio[b]
	MH1160 (OmpR⁻)	MC4100 (OmpR⁺)	
None	0	0	
pMLB952	0	0	
pMLB954	2,396	1,527	1.6
pMLB955	2,109	1,754	1.2
pMLB956	1,918	653	2.9
pMLB957	2,485	1,251	2.0
pMLB958	2,048	1,088	1.9
pMLB959	2,638	1,239	2.1
pMLB960	3,051	1,835	1.7
pMLB961	2,512	1,249	2.0
pMLB962	2,250	1,708	1.3
pMLB963	1,899	832	2.3

[a] Levels of β-galactosidase expressed by different *ompR-lacZ* clones. Units are as defined by Miller (19).

[b] Ratio of β-galactosidase activity of MH1160 (OmpR$^-$) to MC4100 (OmpR$^+$).

EcoRI SmaI BamHI

DNA: ...ACTTGAATTCCCGGGGATCCCGTC...
lacZ reading frame: * Ile Pro Gly Asp Pro Val
(W.T. codon) (9)

FIG. 6. DNA sequence at the *lacZ* gene in pMLB952. The predicted sequence at the linker site in *lacZ* is shown. This sequence was confirmed by DNA sequence analysis of pMLB952. The reading frame of *lacZ* is labeled. The * indicates a stop codon in this frame that includes two-thirds of the *Eco*RI site.

must lie within the DNA sequences from the *Eco*RI site and extend no further into *lacZ* than is consistent with activity, usually taken as codon 26. The DNA distance from the termination codon at the end of the *ompR* structural gene to this first in-frame nonsense codon upstream of *lacZ* was 180 nucleotides. The DNA target within the *lacZ* gene was from the linker-specified codons to codon 26, a distance of 69 nucleotides. The *ompR* gene was 284 codons in length. Therefore, deletions that remove between 180 and 1,101 nucleotides will generate *ompR-lacZ* fusions. In the Lac$^+$ clones examined, deletion sizes ranged from ~319 to ~916 bp. This corresponds to a contribution of from ~52 to ~238 amino acid residues of OmpR to the hybrid OmpR-LacZ proteins (Table 2).

Two of our *ompR-lacZ* fusions showed some phenotypic properties associated with OmpR. Genetic analysis by Hall and Silhavy (16) defined two domains within the *ompR* gene. On the basis of point mutations and insertion mutations in the *ompR* gene, these authors propose that promoter-proximal mutations will confer an OmpF$^-$ OmpC$^-$ phenotype, whereas promoter-distal mutations confer an OmpF$^+$ OmpC$^-$ phenotype. They postulate that a truncated OmpR protein may still function to express *ompF* but not *ompC*. Striking evidence to support this model was provided by the gene fusion on plasmid pMLB954. According to the size of the deletion in this plasmid, ~45 codons were removed from the *ompR* structural gene. However, this fusion conferred an OmpF$^+$ OmpC$^-$ phenotype to a cell that carries a totally negative (OmpF$^-$ OmpC$^-$) *ompR* mutation. At present, we are studying how the *lacZ*-encoded sequence influences the phenotype of this fusion by further in vitro manipulations of this plasmid.

A second unusual phenotype was conferred by plasmid pMLB960. The unexpected result with this plasmid was that in a wild-type OmpR$^+$ cell, this plasmid conferred an OmpR$^-$ phenotype. This dominant negative phenotype can be taken as evidence that the OmpR-LacZ protein was interacting with the wild-type OmpR protein in such a way as to inhibit its action. The position of the fusion joint in plasmid pMLB960 will help us define a functional domain of OmpR that may specify protein-to-protein interactions. Further genetic analysis of this fusion will be required to confirm this model.

In addition, the consistent 1.3- to 2.9-fold difference in expression of the *ompR-lacZ* fusions in the *ompR*$^+$ as compared with the *ompR101* strain backgrounds indicates that OmpR may act to regulate its own synthesis. This regulation may act at the level of transcription or translation and may or may not involve a direct interaction with OmpR. An alternative explanation is that wild-type OmpR protein is interacting with the hybrid OmpR-LacZ protein to reduce the specific activity of the hybrid β-galactosidase. However, this seems unlikely since all of the protein fusions showed this effect. Confirmation of these results will await the construction of transducing phages that carry the various *ompR-lacZ* fusions. These phages will allow us to study the various phenotypes associated with these fusions in lysogens carrying only a single copy of a particular fusion.

One potential use of this technique is to determine the location, size, and orientation of a cloned structural gene. The DNA fragment carrying the wild-type gene would be inserted in pMLB1034 in either orientation with respect to *lacZ*. Provided that neither of these constructs forms a fortuitous in-frame translation start site for *lacZ*, it should be possible to isolate LacZ$^+$ fusions from both clones with our selection procedure. In one orientation, the range of deletion sizes should correspond to the hybrid protein sizes in the manner shown for OmpR-LacZ. Correlating protein molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the deletion size allows placement of the structural gene on the DNA insert. The second orientation may give either a few LacZ$^+$ mutants or none. LacZ$^+$ clones derived from the second plasmid should arise from deletions that fuse *lacZ* to any DNA sequence that can serve as an in-frame translation initiation site. In this case, there may be no correlation between deletion size and apparent molecular weight of the hybrid LacZ monomer. In addition, Lac$^+$ fusions derived from this plasmid may retain the wild-type target gene, whereas in the first orientation (with the exception of an active hybrid gene product), the target gene should be inactive.

The isolation of a series of gene fusions between *lacZ* and a target gene or operon has proven useful. A series of deletions entering a gene from one end provides the basis for rapid mapping of point mutations as well as DNA signals that lie in the gene (20, 21). The ability to isolate bifunctional fusions, i.e., LacZ$^+$ hybrids that retain some of the properties associated with the target gene product, helps to define the role of different protein domains in target gene function (22). Finally, the construction of a LacZ$^+$ protein fusion makes it possible to use the many positive and negative selection techniques for *lac* genetics to isolate mutations in target gene sequence. Without these techniques, it would not have been possible to devise the selection described here. However, by using our selection it will be possible to undertake a systematic study of the properties of *lacZ* protein fusions to any target gene.

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