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Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*

(Recombinant DNA; transposon; insertions; plasmid; hydroperoxidase; complementation; gene interruption)

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SUMMARY

Two genes, *katE* and *katF*, affecting the synthesis of catalase HPII in *Escherichia coli*, have been cloned. The multistep cloning protocol involved: screening for the *tet* gene in a transposon interrupting the genes, selecting DNA adjacent to the transposon, and using it to probe a library of wild-type DNA to select clones from which *katE* and *katF* were subcloned into pAT153. The clones were physically characterized and the presence of the genes confirmed by complementation of their respective mutations. The location of the transposon insertions in the two genes was determined by Southern blotting of genomic digests to further confirm the identity of the cloned genes. A 93-kDa protein, the same size as the subunit of HPII, was encoded by the *katE* plasmid, indicating that *katE* was the structural gene for HPII. A 44-kDa protein was encoded by the *katF* plasmid.

INTRODUCTION

Escherichia coli produces two catalases or hydroperoxidases, labelled HPI and HPII, which differ both in physical properties and in physiological response. HPI is a bifunctional catalase-peroxidase which is active as a tetramer of identical subunits, each 81-kDa, with two protoheme IX groups (Claiborne and Fridovich, 1979). The gene encoding HPI, *katG*, has been mapped (Loewen et al., 1985b) and sequenced (Triggs-Raine et al., 1988) and its expression studied (Finn and Condon, 1975; Hassan and Fridovich, 1978; Richter and Loewen, 1981; Loewen et al., 1985a; Christman et al., 1985). HPII is a monofunctional catalase which is active as a hexamer of identical subunits of 93-kDa, with one heme d group per unit (Loewen and Switala, 1986). The synthesis of HPII is affected by two genes, *katE* at 37.8 min (Loewen, 1984) and *katF* at 59.0 min (Loewen and Triggs, 1984). Unlike HPI, HPII levels did not increase in response to hydrogen peroxide but its levels did increase approximately twenty-fold

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Abbreviations: Ap, ampicillin; bp, base pair(s); HPII, *E. coli* catalase HPII; kb, 1000 bp; Km, kanamycin; LB, see MATERIALS AND METHODS, section a; ^{R}, resistance; SDS, sodium dodecyl sulfate; Tc, tetracycline; UV, ultraviolet; wt, wild type; [ ], designates plasmid carrier state; ::, novel joint (fusion).

TABLE I

Escherichia coli strains, plasmids and bacteriophage used

Strain	Genotype	Source
KL16-99	Hfr *thi recA relA deoB*	CGSC[a]
MP180	HfrH *thi*	Pearson (1972)
SA53	Hfr(R4) *deoA upp udp metB argF relA*	CGSC
RR1	*pro leu rpsL hsdM hsdR endI lacY*	Bolivar et al. (1977)
JK84	*hisS glyA relA argH thi lacY xyl mtl rpsL supE*	CGSC
Q359	P2 lysogen *hsdR hsdM*$^+$	Elledge and Walker (1985)
JM101	*supE thi Δ(lac-proA,B)*[F′*traD*36 *proA*$^+$,*B*$^+$]	Yanisch-Perron et al. (1985)
UM2	*leuB*6 *proC*83 *purE*42 *trpE*28 *his*-208 *argG*77 *ilvA*681 *metA*160 *thi*-1 *ara*-14 *lacY*1 *galK*2 *xyl*-5 *mtl*-1 *azi*-6 *rpsL*109 *tonA*23 *tsx*-67 *supE*44 *malA*38 *xthA katE*2 *katG*15	Loewen et al. (1985b)
UM120	HfrH *thi katE*12::Tn*10*	Loewen and Triggs (1984)
UM122	HfrH *thi katF*13::Tn*10*	Loewen and Triggs (1984)
UM202	HfrH *thi katG*17::Tn*10*	Loewen et al. (1985a)
UM203	Hfr(R4) *deoA upp udp argF relA zij*::Tn*5*	P1(MP180::Tn*5*[b]) × SA53–Km^R*met*$^+$
UM205	*hisS glyA relA argH thi lacY xyl mtl rpsL supE zij*::Tn*5*	P1(UM203) × JK84–Km^R
UM242	*pro leu rpsL hsdM hsdR endI lacY thyA*	Trimethoprim
UM243	*pro leu rpsL hsdM hsdR endI lacY thyA katE12*::Tn*10*	P1(UM120) × UM242–Tc^R
UM244	*pro leu rpsL hsdM hsdR endI lacY thyA katF13*::Tn*10*	P1(UM122) × UM242–Tc^R
UM251	*pro leu rpsL hsdM hsdR endI lacY thyA katE12*::Tn*10* *zij*::Tn*5 argH*	P1(UM205) × UM243–Km^R
UM252	*pro leu rpsL hsdM hsdR endI lacY thyA katF*13::Tn*10* *zij*::Tn*5 argH*	P1(UM205) × UM244–Km^R
UM254	*pro leu rpsL hsdM hsdR endI lacY thyA katE*12::Tn*10 katG*2	P1(UM2) × UM251–*arg*$^+$
UM255	*pro leu rpsL hsdM hsdR endI lacY katG*2 *katE*12::Tn*10 recA*	KL16-99 × UM254–*thy*$^+$
UM257	*pro leu rpsL hsdM hsdR endI lacY thyA katF*13::Tn*10 katG*2	P1(UM2) × UM252–*arg*$^+$
UM258	*pro leu rpsL hsdM hsdR endI lacY katG*2 *katF*13::Tn*10 recA*	KL16-99 × UM257–*thy*$^+$

Plasmid	Antibiotic^R	Source
pBT107	Tc^R	Moyed et al. (1983)
pAT153	Ap^R Tc^R	Twigg and Sherratt (1980)
pMM120	Ap^R	6.2-kb *Bam*HI fragment from λ*katE*::Tn*10* in pAT153
pAMkatE2	Ap^R	5.6-kb *Hin*dIII fragment from λ*katE*6 in pAT153
pAMkatE6	Ap^R	4.8-kb *Cla*I fragment from λ*katE*6 in pAT153
pAMkatE22	Ap^R	4.0-kb *Cla*I fragment from pAMkatE2 in pAT153
pMM122	Ap^R	5.3-kb *Hin*dIII fragment from λ*katF*::Tn*10* in pAT153
pMMkatF1	Ap^R	9.3-kb *Bam*HI fragment from λ*katF*3 in pAT153
pMMkatF2	Ap^R	4.2-kb *Cla*I fragment from pMMkatF1 in pAT153
pMMkatF3	Ap^R	4.2-kb *Cla*I fragment from pMMkatF1 in pAT153

Bacteriophage	Source
λEMBL3	Frischauf et al. (1983)
λkatE::Tn*10*	19.0-kb fragment from UM120 containing Tn*10* in λEMBL3
λkatE6	16.5-kb fragment from MP180 in λEMBL3 complementary to pMM120
λkatF::Tn*10*	19.4-kb fragment from UM122 containing Tn*10* in λEMBL3
λkatF3	17.2-kb fragment from MP180 in λEMBL3 complementary to pMM122

[a] CGSC, Coli Genetic Stock Center, Dr. B. Bachmann, Curator.

[b] A mixed population of Tn*5* in MP180.

during growth into stationary phase and during growth on TCA cycle intermediates (Loewen et al., 1985a). Gene *katF*, but not *katE*, has also been implicated in the protective mechanism against broad spectrum near-UV radiation (Sammartano et al., 1986).

In order to further our understanding of the roles of *katE* and *katF* in the synthesis of HPII, this paper describes their cloning and physical characterization.

MATERIALS AND METHODS

(a) Bacterial strains, plasmids and media

The bacterial strains and plasmids used are listed in Table I. LB medium (Miller, 1972) contained 10 g of tryptone (Difco), 5 g of yeast extract (Difco) and 10 g of NaCl per liter. This medium was supplemented with 0.2% maltose and 10 mM $MgCl_2$ for infection of cultures with bacteriophage. Solid medium was prepared with 1.5% agar. Antibiotics were added as required to the following concentrations: 10 μg Ap/ml and 15 μg Tc/ml.

(b) Catalase activity

Catalase activity on plates was tested by applying a drop of 30% H_2O_2 with a syringe on the edge of the colony. Catalase-positive colonies evolved oxygen bubbles. Catalase activity in liquid medium was determined by the method of Rorth and Jensen (1967) in a Gilson oxygraph equipped with a Clark electrode. One unit of catalase is defined as the amount that decomposes 1 μmol of H_2O_2 in 1 min at 37°C. Liquid cultures were grown at 37°C in LB medium on a shaker bed, monitoring growth with a Klett-Summerson colorimeter using a blue filter (100 Klett unit represented 0.14 mg/ml dry cell weight as determined by weighing culture samples after drying at 100°C and correcting for medium weight). Samples were removed at various times and assayed for catalase activity using 60 mM H_2O_2.

(c) DNA manipulations

Large-scale plasmid preparations were done by the method of Birnboim and Doly (1979). Small-scale plasmid preparations were done by the procedure of Birnboim (1983) and plasmid preparations for the purpose of screening only were done using the direct loading procedure of Maniatis et al. (1982). The digestions of genomic DNA by *Sau*3A, fractionation by centrifugation in a sucrose gradient, and isolation for ligation into λEMBL3 DNA were as described by Maniatis et al. (1982). The packaging kit was purchased from Boehringer Mannheim. The enzymes for DNA analyses were purchased from Boehringer Mannheim and Bethesda Research Laboratories. Restriction enzyme analyses, transformations, ligations, nick-translations, Southern blotting and hybridizations were also done as described by Maniatis et al. (1982). BAL 31 exonuclease digestions were carried out as described by Frey et al. (1984) except that the times of sample removal varied between experiments. After re-precipitation, ligation and transformation, Ap-resistant colonies were selected and screened for catalase activity and the plasmids were characterized.

(d) Synthesis of proteins in maxicells

The proteins encoded by the various plasmids were identified in maxicell extracts as described by Sancar et al. (1979). Cells containing the plasmid were irradiated with UV light to ensure > 99% killing and then labelled with [^{14}C]amino acids. The labelled proteins were analyzed by electrophoresis on 8% polyacrylamide gels containing 0.1% SDS (Laemmli, 1970; Weber et al., 1972). Preparation for fluorography was as described by Boulnois and Timmis (1984).

RESULTS AND DISCUSSION

(a) Cloning of *katE*

Initial attempts to clone *katE* directly from genomic DNA, digested to completion with *Hin*dIII, *Bam*HI, *Eco*RI and *Sal*I, into pAT153 using complementation of the *katE*12::Tn*10* mutation for selection were unsuccessful. The reason for this lack of success will be discussed below. Consequently, a multistep protocol was adopted which is summarized in Fig. 1. A library of genomic DNA from

UM120 (containing *katE*12::Tn*10*) was prepared in λEMBL3 and probed with pBT107 to identify clones containing the Tc^R gene of the transposon. One such clone, *λkatE*::Tn*10*, was selected and its DNA analyzed by restriction nuclease digestion and Southern hybridization of the digests with nick-translated pBT107 to determine the location of the transposon. A DNA fragment adjacent to the transposon generated by *Bam*HI digestion was isolated by cloning into pAT153, giving rise to pMM120. This plasmid was then used to probe a library of genomic DNA from wt MP180 prepared in λEMBL3. One clone, λkatE6, that hybridized with pMM120, was isolated, and characterization of its DNA revealed a pattern of restriction sites that overlapped the pattern surrounding the transposon in λkatE::Tn*10*. Several fragments from λkatE6 were cloned into pAT153, including ones generated by *Cla*I digestion, giving rise to pAMkatE6, and by a partial *Hin*dIII digestion, giving rise to pAMkatE2. These clones were selected by their ability to complement the *katE*12::Tn*10* mutation in UM255, producing oxygen-evolving colonies. Analysis of the DNA revealed common overlapping sequences, and because pAMkatE6 was smaller, containing only 4.5 kb of genomic DNA, it was chosen for expression experiments described below. Subsequently, a smaller plasmid, pAMkatE22, containing only 4.0 kb of genomic DNA, which still complemented *katE*, was isolated by cloning the *Cla*I fragment from pAMkatE2.

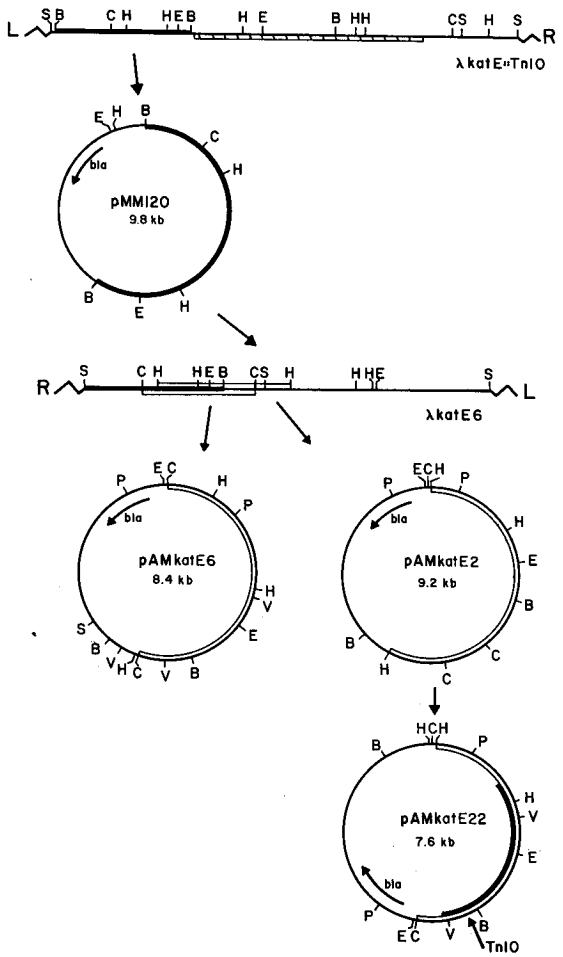

Fig. 1. Strategy for the cloning of *katE*. The hatched segment in *λkatE*::Tn*10* represents the transposon DNA. The solid bar segment adjacent to the transposon represents DNA cloned into pMM120 which was used to probe a genomic library to identify *λkatE*6. The solid bar in *λkatE*6 represents a segment of the insert complementary to pMM120 and the open bars represent segments that were cloned into pAT153 to generate pAMkatE6 and pAMkatE2. The site of Tn*10* insertion is indicated by the arrow in pAMkatE22 relative to the *katE* gene (solid bar). The following abbreviations for restriction enzymes are used: *Bam*HI, B; *Sal*I, S; *Cla*I, C; *Hin*dIII, H; *Eco*RI, E; *Eco*RV, V; *Pst*I, P. The arrows marked *bla* denote the locations and orientation of the β-lactamase gene. In order to simplify the diagram not all sites are shown in every plasmid, just those that are needed for characterization.

The restriction maps of the *katE* clones revealed sites recognized by *Hin*dIII, *Bam*HI, *Eco*RI and *Sal*I in close proximity to the transposon, which is inserted in a location that prevented the production of a functional product from *katE*. These are the same enzymes that were used in the attempts to directly clone *katE*, explaining the lack of success in that approach.

(b) Cloning of *katF*

Attempts to clone *katF* directly from wt genomic DNA using a complementation assay were also unsuccessful and a strategy similar to that described for the isolation of *katE* was employed (Fig. 2). A library of genomic DNA from UM122 (containing *katF*13::Tn*10*) was screened for transposon-containing clones resulting in the isolation of λkatF::Tn*10*. Following characterization of the DNA to locate the transposon, a *Hin*dIII-generated fragment of DNA containing DNA adjacent to the transposon as well as some transposon sequence was cloned to generate pMM122. This plasmid was then used to probe a library of genomic DNA from wt MP180 to identify a clone, labelled λkatF3, which

overlapped the transposon insertion site in λkatF::Tn*10*. A fragment generated by *Bam*HI overlapping the transposon site was cloned into pAT153, giving rise to pMMkatF1, which was selected by complementation of the *katF*13::Tn*10* mutation in UM258. The size of the chromosomal insert was further reduced by sub-cloning a *Cla*I fragment to generate pMMkatF2 (Fig. 2) and pMMkatF3, which differed only in the orientation of the insert DNA but which both complemented *katF*.

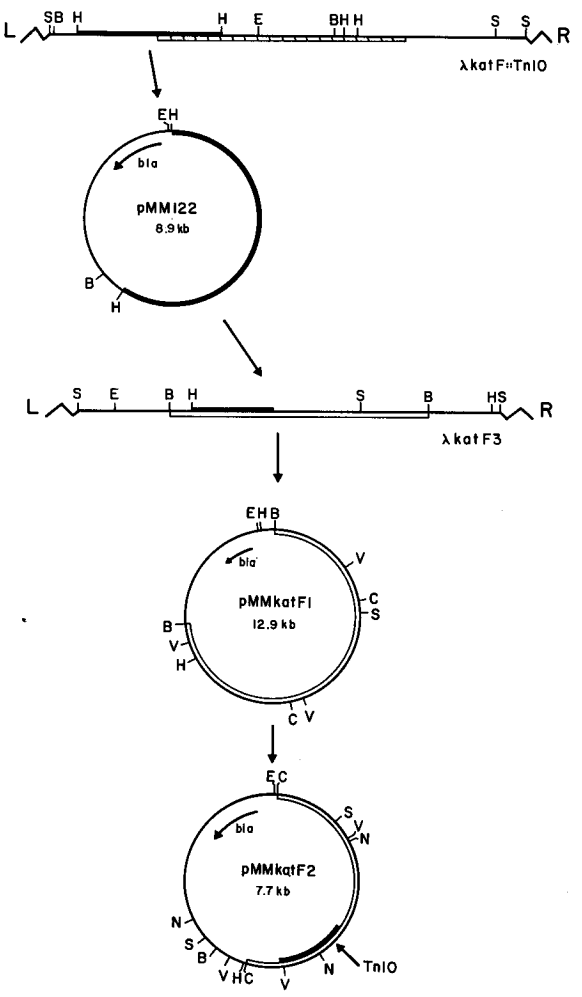

Fig. 2. Strategy for the cloning of *katF*. The hatched segment in λ*katF*::Tn*10* represents the transposon DNA. The solid bar segment overlapping the transposon represents DNA cloned into pMM122 which was used to probe a genomic library to identify λ*katF*3. The solid bar in λ*katF*3 represents a segment of the insert complementary to pMM122 and the open bar represents the segment that was cloned into pAT153 to generate pMMkatF1. The site of the Tn*10* insertion is indicated by the arrow in pMMkatF2 relative to the *katF* gene (solid bar). The other designations are as in Fig. 1 with the addition that *Nru*I is represented by N. In order to simplify the diagrams, not all sites are shown in every plasmid, just those that are needed for characterization.

Unlike the situation with *katE*, none of the restriction enzymes employed in the attempts to directly clone *katF* had recognition sites within pMMkatF2. However, the fragments generated by these enzymes were quite large, which would have reduced the frequency of insertion in pAT153 relative to smaller fragments, possibly explaining why the direct cloning was unsuccessful.

(c) Complementation by *katE* and *katF* clones

In order to confirm that the cloned DNA complemented mutations in the respective genes, the plasmids were transformed into a series of isogenic strains MP180 (wt), UM120 (*katE*::Tn*10*), UM122 (*katF*::Tn*10*) and UM202 (*katG*::Tn*10*). Catalase levels were assayed in mid-log and stationary phase transformed and untransformed cultures, and the results are summarized in Table II. All cells transformed with pAMkatE6, except UM122, exhibited slightly higher levels in mid-log cells and much higher levels (thirty- to one hundred-fold) in stationary-phase cells. The accumulation of HPII was so great in stationary-phase cells containing pAMkatE6 that the crude extracts were visibly green in color compared to the normal amber color. As noted, only

TABLE II

Catalase levels in mid-log and stationary phase cells

Strain	Catalase (units/mg dry cell weight)	
	Mid-log phase	Stationary phase
MP180 (wt)	5.7	58.8
MP180[pAMkatE6]	13.1	1478.0
MP180[pMMkatF2]	14.8	78.2
UM120 (*katE*::Tn*10*)	8.8	17.3
UM120[pAMkatE6]	11.3	1603.0
UM120[pMMkatF2]	15.7	41.3
UM122 (*katF*::Tn*10*)	4.3	17.8
UM122[pAMkatE6]	5.3	12.4
UM122[pMMkatF2]	18.4	60.3
UM202 (*katG*::Tn*10*)	2.0	33.7
UM202[pAMkatE6]	3.9	1060.0
UM202[pMMkatF2]	1.4	54.1

UM122 (containing *katF*::Tn*10*) was unaffected by the *katE*-containing plasmid, indicating that a functional *katF* gene was required for the synthesis of active HPII from the plasmid as well as the chromosome. The effect of pMMkatF2 on catalase levels was not as striking, with only a small, two- to four-fold, increase in catalase being observed in both mid-log and stationary phase cells (Table II). Furthermore, there was no significant difference between complementation of *katE*- and *katF*-containing mutants.

The disadvantage to the data in Table II is that it is not possible to differentiate between HPII and HPI activities, and changes in HPII levels resulting from the plasmids could be masked by HPI. In order to determine what the relative contributions of HPI and HPII were to the changes in Table II, extracts of stationary phase cells were separated on non-denaturing polyacrylamide gels and stained for catalase activity (Fig. 3). Similar patterns were obtained from extracts of mid-log cultures, although the relative band intensities differed somewhat because of the reduced expression of HPII (data not shown). When the changes in HPII levels are viewed qualitatively, the complementation of *katE*::Tn*10* (in UM120) by pAMkatE6 and of *katF*::Tn*10* (in UM122) by pMMkatF2 is very striking in both mid-log and stationary phase cultures. These data confirmed that the very high levels of catalase in stationary-phase cells containing pAMkatE6 observed in Table II were predominantly the result of HPII. The same plasmid even promoted the

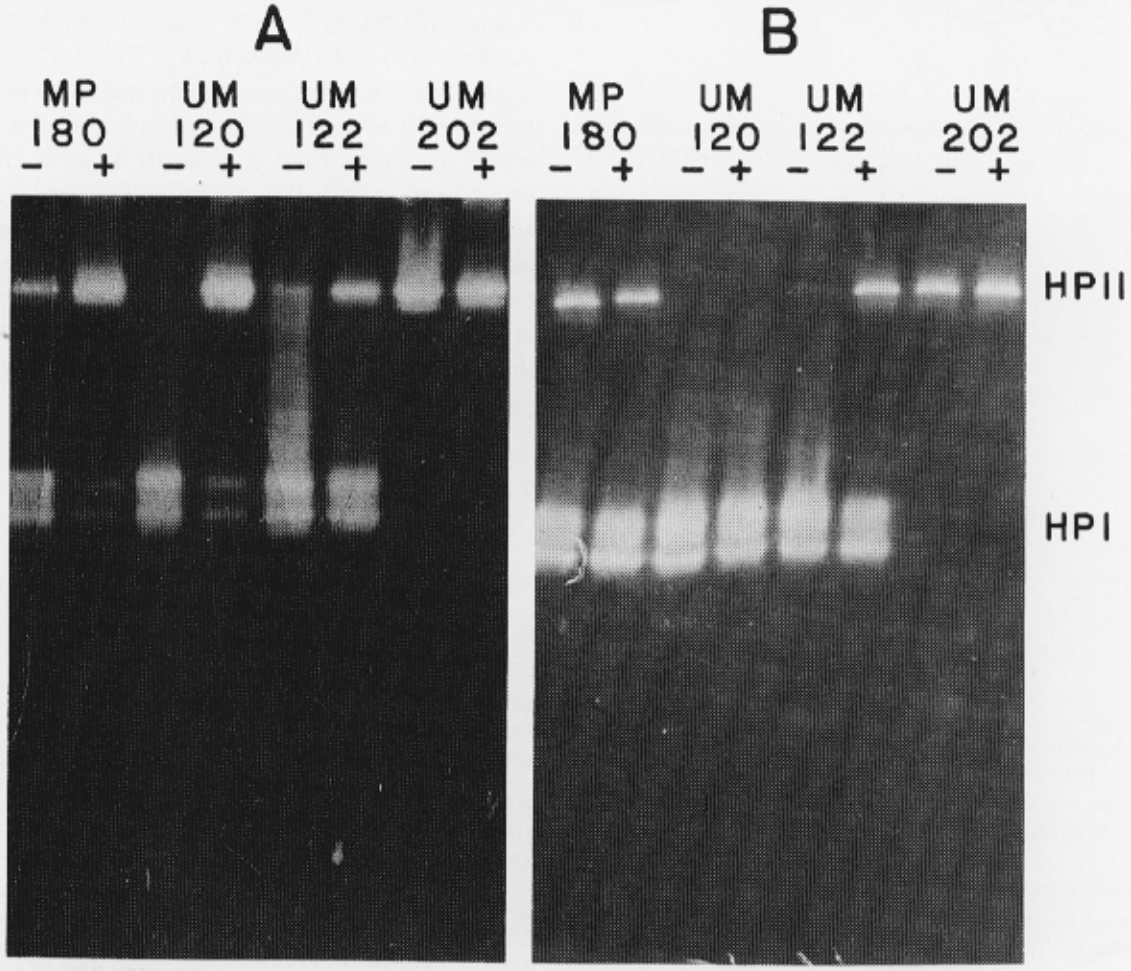

Fig. 3. Visualization of catalase activities following electrophoresis on an 8.5% acrylamide non-denaturing gel (Loewen et al., 1985b) of extracts of various strains, indicated at the top of each lane, grown to stationary phase with and without plasmid. The 15 × 15 cm gels were run at 25 mA until the bromphenol blue dye reached the bottom of the gel. Panel **A**: Strains with (+) and without (−) pAMkatE6 and Panel **B**: Strains with (+) and without (−) pMMkatF2.

synthesis of a small amount of HPII in UM122 (containing *katF*::Tn*10*). The plasmid pMMkatF2 also promoted the synthesis of HPII, but the amounts relative to HPI were smaller, as expected from Table II. Furthermore, pMMkatF2 did not promote the synthesis of any HPII in the *katE*::Tn*10*-containing strain UM120, unlike the reciprocal situation of pAMkatE6 in UM122.

These data suggest that *katE* is the structural gene for the subunit of HPII, while a product of *katF* is involved in enhancing the synthesis of HPII, possibly at the level of *katE* expression. Thus, expression of *katE* on pAMkatE6, albeit at low levels resulting in small amounts of HPII, could occur even in the absence of functional KatF protein, but no HPII was produced when *katE* was disrupted by a transposon, even in the presence of pMMkatF2. Such a conclusion is also consistent with the apparent regulatory role of *katF* in near-UV resistance (Sammartano et al., 1986), wherein it was shown that mutations in *katF* but not *katE* resulted in sensitivity to near-UV radiation. The involvement of *katF* in apparently regulating the expression of two distinct protective pathways suggests that there may be yet another system, in addition to the heat shock, SOS, and OxyR-mediated regulons, that produces a protective response to external stress.

(d) Identification of encoded proteins

The proteins encoded by pAMkatE6 and pMMkatF2 were identified in maxicell extracts (Fig. 4). Plasmid pAMkatE6 produced a 93-kDa protein, the same size as the HPII subunit, confirming the earlier conclusion that *katE* is the structural gene for the HPII subunit. A protein of this size would require a coding region of approximately 2500 bp, or the majority of the chromosomal insert in pAMkatE6. Plasmid pMMkatF2 encoded a 44-kDa protein which is much too small to be the HPII subunit (Fig. 4). The function of the KatF protein in affecting HPII synthesis is currently under investigation.

(e) Confirmation of the chromosomal location of Tn*10* in UM120 and UM122

The transposons in UM120 and UM122 have been extremely valuable in studies on *katE* and *katF*. The strains were initially isolated and characterized as containing mutations in *katE* or *katF* with an associated HPII-deficient phenotype. The transposons facilitated mapping of the two genes and the procedure employed to clone the two genes has utilized the transposons. In order to confirm that *katE* and *katF*, as defined by the transposon insertions, had indeed been cloned, Southern blots of digests of chromosomal DNA from UM120 and UM122 were probed with *katE* and *katF* DNA alongside digests of DNA from wt cells. Specifically, *Cla*I digests of wt MP180 and *katE*::Tn*10*-containing UM120 genomic DNAs were probed with pAMkatE6, resulting in the 4.8-kb fragment in MP180 being replaced by two fragments of 6.3 and 7.8 kb (Fig. 5). These fragments were cumulatively

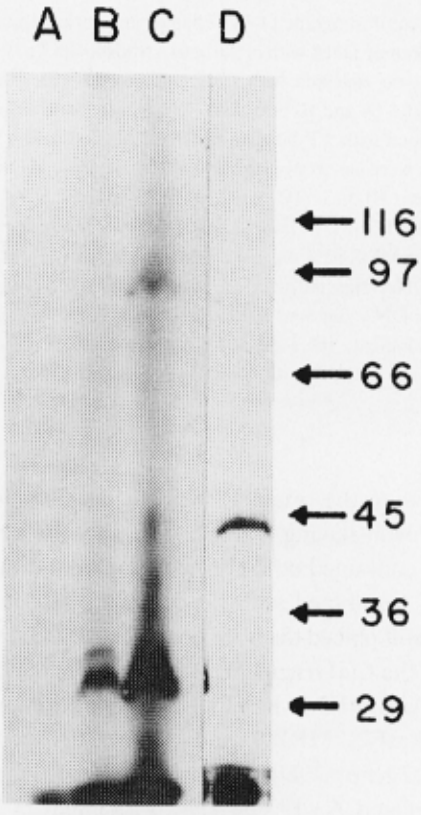

Fig. 4. Maxicell analysis of plasmid-encoded proteins. ^{14}C-labelled polypeptides were analyzed by electrophoresis on a 0.1% SDS-8% polyacrylamide gel at 40 mA for 4 h. Irradiation and fluorography were as described in MATERIALS AND METHODS, section d. Lanes: A, UM255; B, UM255[pAT153]; C, UM255[pAMkatE6]; D, UM258[pMMkatF2]. The numbers along the side indicate the locations of molecular weight standards ($\times 10^3$).

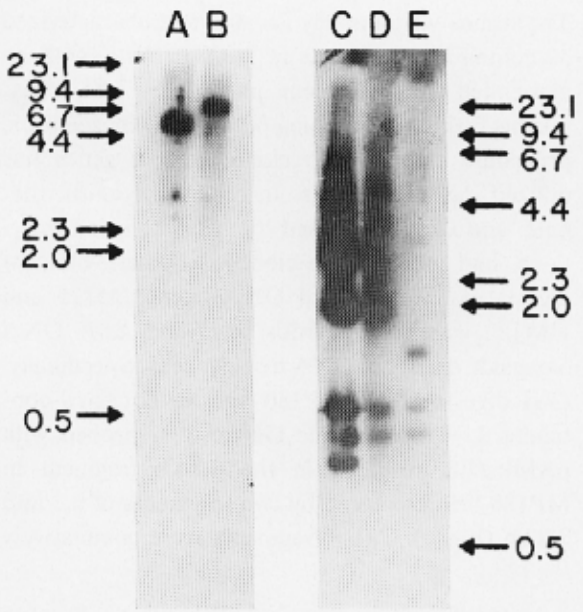

Fig. 5. Autoradiogram of the change in hybridization pattern when genomic DNA with or without a transposon Tn*10* insertion in *katE* was digested with *Cla*I and probed with ^{32}P-labelled pAMkatE6 (**A** and **B**) or in *katF* was digested with *Cla*I + *Nru*I and probed with ^{32}P-labelled pMMkatF2 (**C**–**E**). The following samples were electrophoresed on a 0.8% agarose gel at 80 V for 2 h (**A** and **B**) or 3 h (**C**–**E**): **A**, MP180 genomic DNA digested with *Cla*I; **B**, UM120 (*katE*::Tn*10*) genomic DNA digested with *Cla*I; **C**, pMMkatF2 digested with *Cla*I + *Nru*I; **D**, MP180 genomic DNA digested with *Cla*I-*Nru*I; **E**, UM122 (*katF*::Tn*10*) genomic DNA digested with *Cla*I + *Nru*I. The numbers along the side indicate the sizes in kb of fragments generated by the digestion of λ DNA with *Hin*dIII.

larger than the original fragment because, with the transposon having a single *Cla*I site, the new fragments contained both chromosomal and transposon DNA. Combined with the mapping data in Fig. 1, this result placed the transposon just 1.2 kb from one end of the *Cla*I fragment, as indicated in the diagram of pAMkatE22 in Fig. 1. For probing with pMMkatF2, MP180 and *katF*::Tn*10*-containing UM122 genomic DNAs were digested with a mixture of *Cla*I and *Nru*I. The 1.9-kb fragment in MP180 DNA was replaced by a 1.6-kb fragment (Fig. 5). This fragment would contain only 80 bp of transposon DNA and the remainder of the original fragment, 0.3 kb, was sufficiently small that it was lost from the bottom of the gel. Combined with the mapping data in Fig. 2, the transposon insertion site was concluded to be 0.4 kb from the *Nru*I site in pMMkatF2 as indicated by the arrow (Fig. 2). The fact that the transposons previously mapped in the respective genes were found to interrupt fragments from both of the clones confirmed that the respective genes had been cloned and allowed a definition of the transposon insertion sites. Furthermore, the restriction maps of λkatE6 and λkatF3 are in close agreement with the *E. coli* genomic map in the 37 and 59 min regions, respectively, deduced by Kohara et al. (1987).

The transposon sites were useful in defining the limits of *katE* and *katF* in conjunction with BAL 31 deletion mutants pAMkatE6 and pMMkatF2. In pAMkatE6, a BAL 31 digest was initiated at the unique *Sal*I site, and removal of more than 0.7 kb from the chromosomal insert, up to but not including the *Eco*RV site adjacent to the transposon site, inactivated the *katE* function. Approximately 2.5 kb are required to encode the 93-kDa HPII subunit, and its location on pAMkatE22 is indicated in Fig. 1. In pMMkatF2, deletion of only 0.4 kb of the chromosomal insert from the *Hind*III site, up to but not including the *Eco*RV site (Fig. 2), inactivated the *katF* function. A minimum of 1.3 kb is required to encode a transcription unit containing KatF protein, and the distance between the transposon and *Eco*RV sites is very similar to this distance. The probable location of *katF* in pMMkatF2 is indicated in Fig. 2.

(f) Conclusions

(**1**) The *E. coli* genes, *katE* and *katF*, involved in catalase HPII synthesis were isolated on 4.0 kb fragments in the plasmids pAMkatE22 and pMMkatF2, respectively. (**2**) *katE* is the structural gene for the subunit of catalase HPII, encoding a 93-kDa protein. (**3**) *katF* encodes a 44-kDa protein that is required for the expression of *katE*.

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