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Nucleotide sequence of *katF* of *Escherichia coli* suggests *KatF* protein is a novel σ transcription factor

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ABSTRACT

The *katF* gene of *Escherichia coli* has been sequenced revealing a 1086 base pair open reading frame from which the sequence of a 362 amino acid protein has been deduced. The direction of transcription of *katF* was confirmed by expression of the gene cloned in both directions behind a T7 promoter. The KatF protein expressed in vitro migrates with an apparent size of 42 kDa. Comparison of the *katF* sequence to the sequence of *rpoD*, which encodes the sigma subunit of RNA polymerase, revealed a 181 bp region with 65% homology and a 38 bp segment that was 87% homologous. A 62 amino acid region of the predicted KatF protein sequence was found to be 85% homologous to the corresponding sequence of σ^{70}, including a segment implicated in core polymerase binding. Homology was also observed with the heat shock regulatory protein encoded by *htpR*.

INTRODUCTION

Escherichia coli produces two different catalases, HPI (1) and HPII (2), for protection against the damaging effects of hydrogen peroxide arising from cellular metabolism and from external sources. The levels of catalase HPI increase in response to external H_2O_2 (3,4) in a process mediated by OxyR protein (5). The second catalase, HPII, does not respond to external hydrogen peroxide but growth into stationary phase or growth on TCA cycle intermediates caused a 20 fold increase in its levels (6). The roles of the two enzymes may therefore be different despite similar catalytic properties with HPII being part of a system to protect the cell against oxidative damage when the cells are not actively growing. The structural genes for the two enzymes have been mapped with *katG*, encoding HPI, mapping at 89.2 min (7) and *katE*, encoding HPII, mapping at 37.8 min (8). The expression of *katE*, but not *katG*, was found to be dependent on a third gene,

katF, which mapped at 59.0 minutes (9) and which encoded a 42 kDa protein (10). The role of KatF protein in enhancing the expression of *katE* was not defined.

In addition to its initial characterization as an enhancer of catalase HPII synthesis, the *katF* gene product has more recently been implicated in protection against broad-spectrum ultraviolet radiation (11) and in the expression of *xthA*, which encodes exonuclease III, another repair enzyme (12). KatF protein may therefore be an important factor in turning on expression of a series of genes required for protection against external stresses. This report describes the sequence of *katF* and the finding of extensive homology with the sequence of *rpoD*, encoding the sigma subunit of RNA polymerase. A model involving KatF protein acting as a new sigma factor controlling a regulon of protective genes is described.

METHODS

Media, strains and plasmids The strains JM101 (13), HB101 (14) and UM258 *pro leu rpsL hsdM hsdR katG2 katF13::Tn10 recA* (10) were grown in LB medium (15). Fragments of *katF* were cloned (16) into M13 (17) for sequencing (18, 19). Fragments of *katF* were cloned into pT7-5 (20) for expression by T7 RNA polymerase. Deletions of *katF* were generated for sequencing using *Sau*3AI and *Bam*HI (21).

Expression of *katF* A *Hin*dIII-*Eco*RI fragment from plasmids pMMkatF2 and pMMkatF3 containing *katF* was cloned into pT7-5 generating pT7F2 and pT7F3 in which the *katF* gene was in opposite orientations relative to the T7 promoter. These plasmids were used to transform a strain containing the plasmid pGP1-2. The double transformants were heat shocked by transferring a cell culture from 30°C to 42°C as described (20). After 30 minutes, the cells were collected and lysed by sonication. Extracts of the crude extract were separated by SDS polyacrylamide gel electrophoresis (22,23) and the protein bands visualized with Coomasie blue dye. The proteins were also labelled with a [^{14}C]amino acid mixture and the labelled protein bands were localized by autoradiography (24).

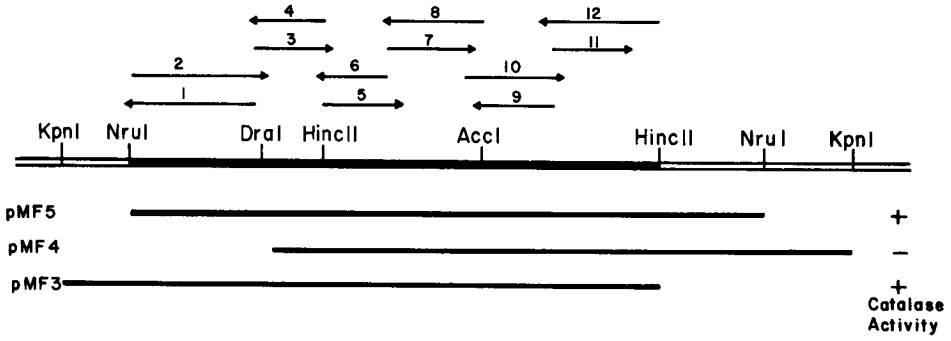


Figure 1. Localization of *katF* and sequencing strategy. The three clones, pMF3, pMF4 and pMF5 containing the indicated segments of the *Kpn*I fragment in an M13+ phagemid, were constructed and tested for complementation of the *katF* mutation in UM258. The blocked in region between the *Nru*I and *Hinc*II sites was found to be required for *katF* activity and was sequenced. The clones, numbered 1 to 12, were isolated in M13mp18 and M13mp19 for sequencing.

RESULTS

Cloning of *katF* A two step procedure using transposon Tn*10* insertion mutants was used to clone *katF* resulting in its localization on a 4.1 kb fragment which complemented *katF* mutations (10). A 42 kDa protein was expressed from this plasmid and because this would require only 1.5 kb of coding sequence, a further subcloning of the gene was attempted before undertaking a sequence determination. This resulted in *katF* being localized on a 2.3 kb fragment generated by *Kpn*I digestion (Figure 1). Smaller subclones of the *Kpn*I fragment were isolated and screened for their ability to complement *katF* mutations in UM258. As shown in Figure 1, a 1.5 kb DNA segment extending from a *Nru*I site to a *Hinc*II site was required for complementation.

Nucleotide sequence of *katF* Having reduced the size of the *katF* clone, sequence analysis was undertaken employing the strategy shown in Figure 1 to sequence both strands giving rise to a sequence of 1482 base pairs (Figure 2). A single open reading frame of 1086 base pairs was found which encodes a protein of 362 amino acids. The predicted molecular weight of this protein,

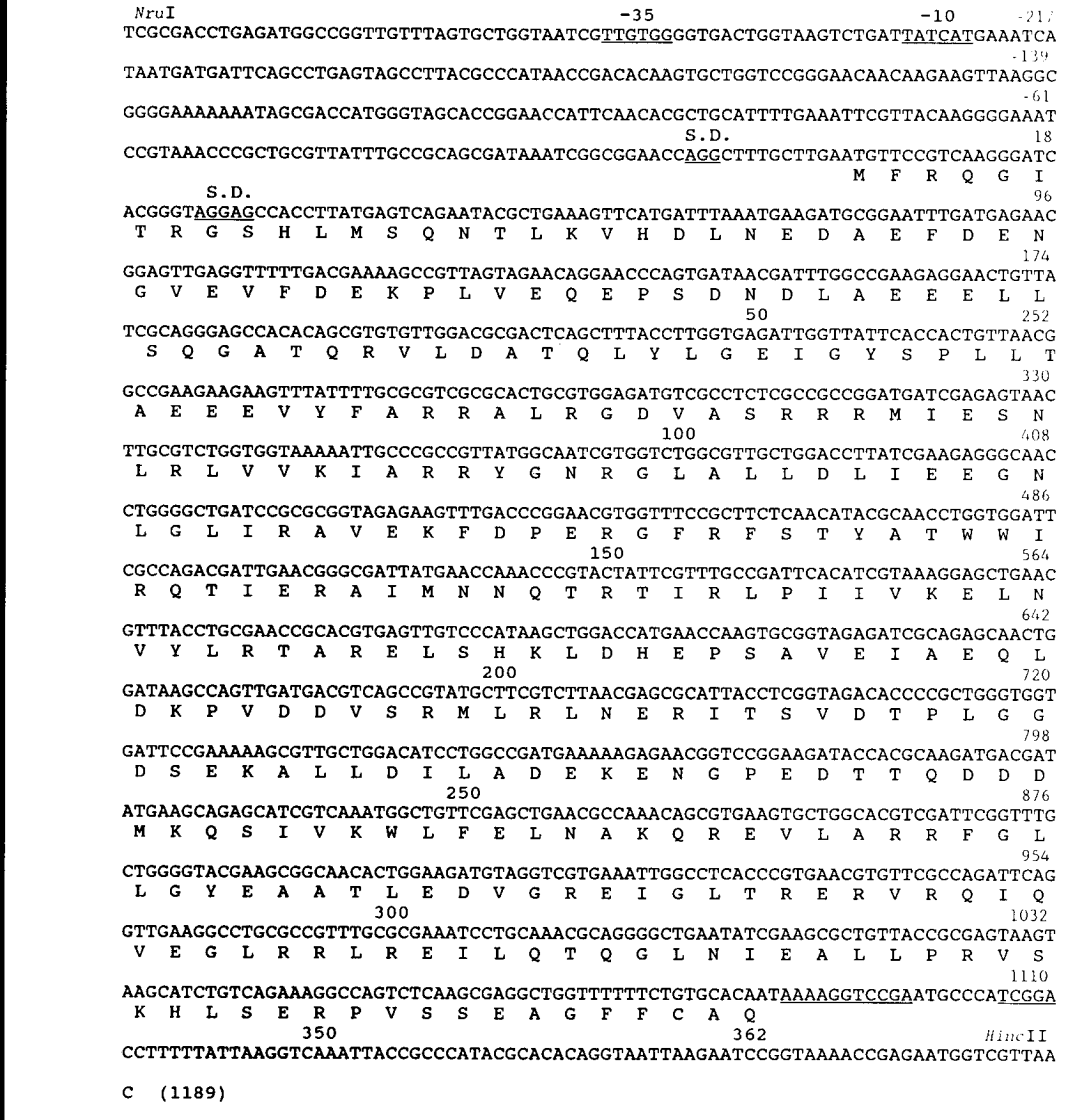


Figure 2. Nucleotide sequence of the *katF* gene and predicted amino acid sequence. Potential -35, -10, Shine-Dalgarno and terminator sequences are indicated.

41.5 kDa, is very similar to the 42 kDa size of KatF protein determined by gel electrophoresis. The amino acid composition predicted by the DNA sequence is summarized in Table 1.

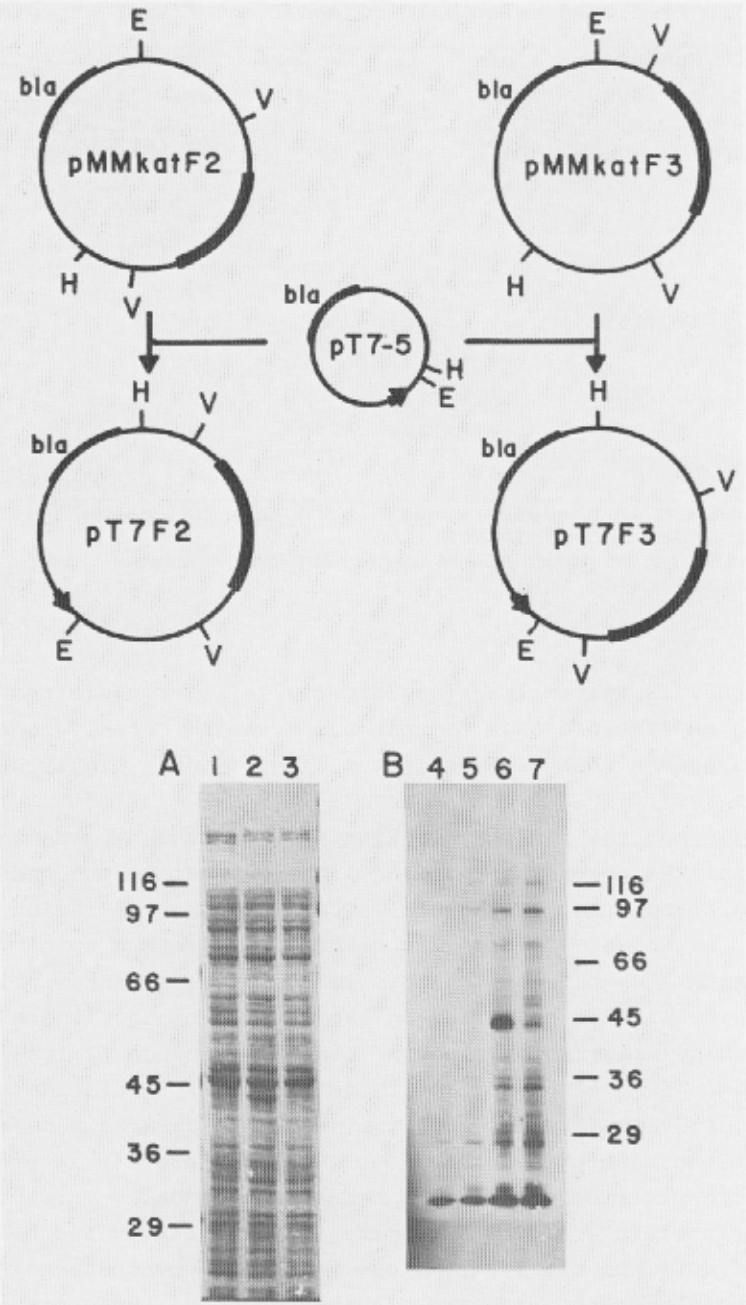
Table 1. Predicted amino acid composition of KatF protein

Amino acid	Number of residues[a]	% of total[b]	Amino acid	Number of residues[a]	% of total[b]
Ala	25	6.9 (7.1)	Leu	47 (46)	12.9 (13.1)
Arg	37 (35)	10.2 (10.0)	Lys	13	3.6 (3.7)
Asn	13	3.6 (3.7)	Met	6 (5)	1.7 (1.4)
Asp	23	6.4 (6.6)	Phe	11 (10)	3.0 (2.8)
Cys	1	0.3 (0.3)	Pro	11	3.0 (3.1)
Gln	17 (16)	4.7 (4.6)	Ser	18 (17)	5.0 (4.8)
Glu	41	11.3 (11.7)	Thr	18 (17)	5.0 (4.8)
Gly	22 (20)	6.1 (5.7)	Trp	3	0.8 (0.9)
His	6 (5)	1.7 (1.4)	Tyr	7	1.9 (2.0)
Ile	20 (19)	5.5 (5.4)	Val	23	6.3 (6.6)

[a] The numbers in parentheses are the number of residues in the shorter 350 amino acid KatF.
[b] The numbers in parentheses represent the % composition in the shorter 350 amino acid KatF.

Noteworthy is the excess of acidic (17.7%) over basic residues (13.8%) and the presence of only one cysteine. Analysis of codon usage suggests that KatF is in the category of a highly expressed protein (25).

Potential control regions flanking *katF* There is only one potential RNA polymerase binding site upstream of the open reading frame. The -10 region, TATCAT, and the -35 region, TTGTGG, begin at -229 and -254 respectively with a spacing of 19 base pairs. Whether or not this is the actual transcription start site will be clarified in later experiments. There is a short Shine-Dalgarno or ribosome binding sequence (26) AGG thirteen base pairs upstream of the potential initiation ATG codon. The actual start site assignment is uncertain however, because the methionine at residue 13 of the protein is preceeded by a stronger ribosome binding sequence of AGGAG. This potential shorter protein would be 350 amino acids in length and have a size of 40.1 kDa still very close to the observed size of 42 kDa. Based solely on the ribosome binding sequence, the shorter protein should be the predominant form but a clear definition of


E
bla
V
pMMkatF2
H
V
E
V
bla
pMMkatF3
H
V
bla
pT7-5
H
E
H
V
bla
pT7F2
E
V
H
bla
V
pT7F3
E
V
A 1 2 3
B 4 5 6 7
116
97
66
45
36
29
116
97
66
45
36
29

the start site will have to await N-terminal sequence analysis of the protein.

Following the putative termination codon TAA there is an element of two fold symmetry with a predicted stability of -21.0 kcal/mol which is followed by a run of four Ts indicative of a strong transcription termination site. In fact three of these T's could be included in the loop structure increasing its predicted stability to -24.6 kcal/mol. No other potential open reading frames were found in the flanking regions

Direction of transcription of *katF* In order to confirm the direction of transcription of *katF*, it was cloned in both orientations downstream from a T7 promoter in the vector pT7-5 (Figure 3). The pT7 derivatives were then used to transform a strain containing the plasmid pGP1-2, encoding T7 RNA polymerase downstream from the lambda promoter P_L controlled by the heat sensitive cI857 repressor. Heat shock of the doubly transformed strain resulted in production of T7 RNA polymerase from pGP1-2 which in turn transcribed DNA downstream from its promoter on the pT7 plasmids. Of the two plasmids containing *katF*, only pT7F2 yielded a significant band of ^{14}C-labelled protein with an apparent size of 42 kDa (Figure 3B). The over-production of KatF protein in this system was great enough that a band of protein was evident in crude extracts stained only with Coomassie blue (Figure 3A). The expression of KatF from pT7F2 is consistent with the direction of expression implied by the open reading frame in the nucleotide sequence.

Figure 3. Construction of clones with *katF* adjacent to a T7 promoter and analysis of expressed proteins. The 4.0 kb fragment generated by *Hin*dIII and *Eco*RI from either pMMkatF2 or pMMkatF3 was transfered into pT7-5 generating pT7F2 and pT7F3. Proteins expressed from these plasmids following heat shock were separated by SDS polyacrylamide gel electrophoresis and stained with Coomasie brilliant blue (A) or subjected to autoradiography (B). Extracts from cells containing pMMkatF2, pT7F2 and pT7F3 were run in lanes 1,2 and 3 respectively. Extracts from cells without plasmid, with pT7-5, pT7F2 and pT7F3 were run in lanes 4, 5, 6 and 7 respectively. The location and sizes (X 10^{-3}) of various molecular weight markers are indicated alongside the gels.

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htpR     207  GGGCTATGGCCTGCCACAGGCGGATTTGATTCAGGAAGGTAACATCGGCCTG
              *** *** *   *  ***  * **  * ** ** *** * ** ***
katF     366  CAATCGTGGTCTGGCGTTGCTGGACCTTATCGAAGAGGGCAACCTGGGGCTG
              *** *****  **  *** ** ***** **  * ** ****** * ** ***
rpoD    1709  CAACCGTGGCTTGCAGTTCCTTGACCTGATTCAGGAAGGCAACATCGGTCTG

htpR     259  ATGAAAGCAGTGCGCCGTTTCAACCCGGAAGTGGGTGTGCGCCTGGTCTCCT
              **    ** **       **  ********   *** * *** *     * *
katF     418  ATCCGCGCGGTAGAGAAGTTTGACCCGGAACGTGGTTTCCGCTTCTCAACAT
              **    ***** ** ** ** **       ******* *   ***** ** *
rpoD    1761  ATGAAAGCGGTTGATAAATTCGAATACCGCCGTGGTTACAAGTTCTCCACCT

htpR     311  TCGCCGTTCACTGGATCAAAGCAGAGATCCACGAATACGTTCTGCGTAACTG
               ***       *****         ***  *        ** **    *
katF     470  ACGCAACCTGGTGGATTCGCCAGACGATTGAACGGGCGATTATGAACCAAAC
              **************** ** *** ****    **  * **   * * **  *
rpoD    1813  ACGCAACCTGGTGGATCCGTCAGGCGATCACCCGCTCTATCGCGGATCAGGC

htpR     363  GCGTATCGTCAAAGTTGCGAC
               ****   *     *  ***
katF     522  CCGTACTATTCGTTTGCCGAT
               ** ** ** *** * *** *
rpoD    1865  GCGCACCATCCGTATTCCGGT
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Figure 4. Comparison of the nucleotide sequence of part of *katF* with portions of *rpoD* and *htpR*. Common bases are indicated by an asterisk. The region from 460 to 497 of *katF* which is 87% homologous with a portion of *rpoD* is indicated with the solid line.

Comparison of the *katF*, *rpoD* and *htpR* sequences When the sequence of *katF* was compared to other known sequences, a significant region of homology was found with the sequence of *rpoD*, the gene that encodes the RNA polymerase sigma factor σ^{70}. As shown in Figure 4 the sequence of *katF* from 366 to 542 is 66% homologous to a portion of *rpoD* (27) and a smaller 38 bp sequence from 460 to 497 was found to be 87% homologous. Homology was also found with *htpR* (28) but to a lesser extent (Figure 4). This observed homology with the genes of two known sigma factors clearly suggesteda similar role for the product of katF.

Comparison of the amino acid sequences of KatF, HtpR and σ^{70} A comparison of the deduced amino acid sequence of KatF protein with the sequences of σ^{70} and HtpR proteins (Figure 5) confirmed that there was substantial homology at the protein level as well as at the nucleotide level. Including conservative replacements, there is 46% homology for the whole of KatF protein with a 362

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katF     1  MFRQGITGRSHLMSQNTLKVHDLNEDAEFDENGVEVFDEKPLVEQEPSDNDLAE
                  =  *       * =*       *    *                =     =
rpoD    29  DHLPEDIVDSDQIEDIIQMINDMGIQVMEEAPDADDLMLAENTADEDAAEAAAQ

htpR     1  MADKMQSLALAPVGGLDSYIRAANAWPMLSADEERALAEKLHYHGDLEAAKTLI
                =      *       *       =*=*=*==    = *     ==*    * *=
katF    55  EELLSQGATQRVLDATQLYLGEIGYSPLLTAEEEVYFARRDLR-GDVASRRRMI
                     =   =    *=* =*=   === = =*  =*=           ** =*
rpoD    83  VLSSVESEIGRTTDPVRMYMREMGTVELLTREGEIDIAKRIED KARRAKKEMV
                                                          371
              [-------------------------------REGION 2------------
htpR    55  LSHLRFVVHIARNYAGYGLPQADLIQEGNIGLMKAVRRFNPEVGVRLVSFAVHW
             = == == === =    ==    === ===*=**== *= == = =  **=  =
katF   108  ESNLRLVVKIARRYGNRGLALLDLIEEGNLGLIRAVEKFDPERGFRFSTYATWW
            = ======* ==***= ====   ==== ===*==**==*===*  ==**=======
rpoD   381  EANLRLVISIAKKYTNRGLQFLDLIQEGNIGLMKAVDKFEYRRGYKFSTYATWW

            -------------------------------]           [------------
htpR    109 IKAEIHEYVLRNWRIVKVATTKAQRKLFFNLRKTKQRLGWFNQDEV-EMVAREL
            =*  =    **   = ***           *  =  *         *     *=  =
katF    162 IRQTIERAIMNQTRTIRLPIHIVKELNVYL-RTARELSHKLDHEPSAVEIAEQL
            === = = =  = ====*=*=**  *=     =  = *   *  ==*  =*== *
rpoD    435 IRQAITRSIADQARTIRIPVHMIETINKLN-RISRQMLQEMGREPTPEELAERM

            ------REGION 3-------------------------]
htpR    162 GVTSKDVREMESRMAAQDMTFDLSSDDDSDSQPMAPVLYLQDKSSNFADGIEDD
                 ==  =  =*     * =     ==*           =             = ==
katF    215 DKPVDDVSRM-LRLNERITSVDTPLGGDSEK------ALLDILADEKENGPEDT
              = = * ** =**     =**==*= = *          == * =     = =*
rpoD    488 LMPEDKIRKV-LKIAKEPISMETPIGDDEDS------HLGDFIEDTTLELPLDS

                          [-------------------REGION 4----------
htpR    216 NWEEQAANRLTDAMQGLDERSQDIIRARW-LDEDNKSTLQELADRYGVSAERVR
              **     *   *  =  *  ***  =  =      == **    =** ====
katF    262 TQDDDMKQSIVKWLFELNAKQREVLARRFGLLGYEAATLEDVGREIGLTRERVR
                * **       =  = =*   ==  ===*      ===*===*   *====*=
rpoD    535 ATTESLRAATHDVLAGLTAREAKVLRMRFGIDMNTDYTLEEVGKQFDVTRERIR

            ------------------------]
htpR    269 QLEKNAMKKLRAAIEA (284)
            =*    ***==  *
katF    316 QIQVEGLRRLREILQTQGLNIEALLPRVSKHLSERPVSSEAGFFCAQ (362)
            ==    ==*==    *  =
rpoD    589 QIEAKALRKLRHPSRSEVLRSFLDD (613)
```

Figure 5. Comparison of the predicted amino acid sequence of KatF with portions of the sequences of σ^{70} and HtpR. Identical amino acids are indicated by = and conservative matches are indicated by *. The regions 2, 3 and 4 are regions that have been conserved in several different sigma factors (30). Alignment of the sequences is similar to the alignment in reference 29 with 244 amino acids of σ^{70} being omitted at the diagonal slash (residues 127 to 370).

amino acid portion of σ^{70}. If just the middle portion of KatF is considered, the region from residue 104 to 184 has 65% identical matching and 85% total matching, including conservative replacements, with residues 375 to 455 of σ^{70}. Two fourteen-

amino acid segments in this region are 100% matching. Homology was also evident between HtpR and KatF but the extent was not as great, there being only 63% total matching between residues 103 and 184 of KatF and an equivalent portion of HtpR.

A number of sigma factors from different bacteria have been compared (29, 30) revealing three and possibly four domains that have been conserved. KatF protein also contains the conserved regions 2,3 and 4 and even shows some homology in region 1 (Figure 5) providing further substantiation for its role as a sigma factor. Regions 2.4 (amino acids 435 to 452 in σ^{70}) and 4 have been implicated in interactions with the -10 and -35 sequences respectively of promoters (30). The 67% and 61% homology in regions 2.4 and 4 respectively between σ^{70} and KatF is consistent with KatF controlling expression of a different set of promoters.

More recently a region including amino acids 361 to 390 in σ^{70} was identified as being crucial for core binding (31). A comparison with the corresponding region in KatF, amino acids 88 to 117, reveals no homology in the first 15 amino acids but 80% homology in the second 15, amino acids 376 to 390 in σ^{70} corresponding to 103 to 117 in KatF. It may therefore be only the latter half of this region that is involved in core binding.

DISCUSSION

katF was initially identified as a gene required for the expression of *katE* (7) and when it was later found that a diffusible gene product was encoded by *katF* (10), a positive regulatory role for the protein was suggested. A mechanism for this positive effect on *katE* transcription can now be proposed on the basis of the close sequence relationship between katF and known sigma factors. While other types of regulatory mechanisms cannot be eliminated, the sigma factor-like sequence of KatF protein argues strongly that it affects the expression of *katE* by modifying the affinity of RNA polymerase for the *katE* promoter.

katF has also been shown to affect the expression of other genes involved in the protection of the cell against the harmful effects of near-UV radiation. Mutants lacking *katF*, but not *katE*

or *katG*, have been found to be more sensitive to broad spectrum near-UV radiation (11). The minor role played by catalase in protecting the cell against near-UV damage (32) suggested the involvement of other genes, one candidate being *xthA* which encodes exonuclease III. *xthA* mutants have been shown to be more sensitive to near-UV radiation (33) and its expression is affected by *katF* (12). Hence, the role of *katF* in affecting resistance to near-UV radiation probably lies in its activation of expression of *xthA* and possibly other genes encoding DNA repair enzymes. The transcription start site for *xthA* (35) was preceeded by a sequence that did not contain a -35 region suggesting that the affinity of RNA polymerase had been modified, possibly by an activating protein (35). Rather than an activating protein, it is KatF protein acting as a sigma factor which has modified the sequence preference of the polymerase to recognize the *xthA* site.

The two genes controlled by *katF*, *katE* and *xthA*, map very close to one another on the chromosome, at 37.8 and 38.2 min respectively (8) raising the possibility that they are expressed as part of the same operon (12). Assuming that 1 min includes 45,000 bp (34), the distance between the two genes is approximately 18,000 bp, an extremely long segment to be transcribed into one mRNA. Furthermore, both *katE* and *xthA* are expressed well when separately cloned (10,35), indicating that both genes have promoters in close proximity to their coding regions. As a potential sigma factor, KatF protein is therefore activating at least two promoters, one to enhance catalase production to reduce H_2O_2 levels and a second to enhance synthesis of an enzyme required for the repair of DNA damage. Furthermore, with sigma factors being few in number in *E. coli*, it is not unreasonable to expect KatF to be involved in the transcription of other genes involved in protection or repair roles.

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