Effects of the *unc*I gene on expression of *unc*B, the gene coding for the a subunit of the F_1F_0 ATPase of Escherichia coli

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Abstract The eight genes coding for the subunits of the *E. coli* F_1F_0 ATPase are preceded by a gene, designated *unc*I. A homologous gene, or a gene coding for an analagous protein, is found preceding the ATPase genes of several microorganisms. No function for the I gene has been described. Using *lac* fusions to measure gene expression in vivo, we tested the effects of deleting *unc*I on the expression of the adjacent gene *unc*B, which codes for the a subunit of the F_0 sector of the ATPase. Deleting *unc*I reduced the expression of three *unc*B′-′*lac*Z fusion genes in vivo, but had no effect on the expression of two *unc*B′-′*lac*Z fusion genes containing a relatively smaller amount of the *unc*B coding region. The *unc*I deletion also reduced the relative synthesis of the a subunit in vitro. The I gene therefore appears to specifically affect the expression of *unc*B or the synthesis of the a subunit at some step after translational initiation of *unc*B.

Key words: Proton-translocating ATPase; *E. coli*; ATPase gene

1. Introduction

The F_1F_0 ATPase of *E. coli* is a large multimeric membrane-bound enzyme which catalyzes the synthesis or hydrolysis of cellular ATP, and couples that reaction to the movement of protons across the cytoplasmic membrane. The complex consists of two large sectors. The intrinsic membrane-bound F_0 sector, consisting of three subunits - a, b, and c - allows protons to flow through the membrane. The peripheral F_1 sector is bound to the F_0, consists of five subunits - $\alpha, \beta, \gamma, \delta$, and ε -and contains the catalytic sites for ATP synthesis or hydrolysis (for recent review, see [1]). The genes coding for the ATPase are encoded in an operon, designated *unc*, located at 84 minutes in the *E. coli* chromosome. In addition to the eight genes coding for the subunits of the ATPase, this operon contains a ninth gene, *unc*I, which precedes the structural genes [2]. A homologous gene or a gene for an analagous protein is found at the start of the operons encoding the ATPase genes of a variety of microorganisms [3–12]. Despite the evolutionary conservation of this gene, no function has yet been attributed to it. The I gene of *E. coli* has been cloned, expressed, overexpressed, and mutated with no apparent effects on ATPase function [13–18]. In this study, we measured the effects of deleting or inactivating *unc*I on the expression of downstream genes, specifically the *unc*B and *unc*E genes, encoding the a and c subunits of the F_0.

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2. Materials and methods

2.1. Bacterial strains and plasmids

All experiments were carried out in strain LE392Δ[*unc*I-*unc*C] [19–20]. Plasmids pKS102, pDKWH103, pKS103, pKS104, carrying the *unc*B′-′*lac*Z fusion genes, and pKS105, carrying the *unc*E′-′*lac*Z fusion gene, have all been described previously [21]. The fusion gene in pDKWH107 was constructed by first cloning the 617 base pair *Bam*HI fragment within *unc*B into M13mp18 to create pDKWH1301. Single-stranded template DNA was mutagenized to create an additional *Bam*HI site 111 base pairs downstream from the *Bam*HI site at the promoter-proximal end of the 617 base pair fragment. The resultant double-stranded construction was named pDKWH1301B. The 111 base pair *Bam*HI fragment in this construction was cloned from pDKWH1301B into pDKWH103 [21], and the resultant plasmid, designated pDKWH107, contained the *unc* promoter, *unc*I, and 199 base pairs of *unc*B fused in-frame to *lac*Z. When present in a plasmid, these fusion constructions are designated by the prefix p. When present in λ, they are designated by the prefix λp.

The *unc*I gene in each of these fusion plasmids was mutated in two ways. One set of *unc*I mutants was made by digesting each plasmid with *Hin*dIII and religating, thus deleting the 199 base pair *Hin*dIII region in *unc*I from each (see Fig. 1). These mutant plasmids were given the same designation along with the suffix A. In addition to the 'A' mutants, a second set of *unc*I mutants was made by mutating this 199 base pair *Hin*dIII fragment to add two stop codons and a frameshift mutation. The 199 base pair *Hin*dIII fragment was cloned into M13mp19 to create pDKWH1305. Oligonucleotide-directed in vitro mutagenesis was performed using a mutant primer, DH632 (5′-GCCTCAAA-GACCCCTTCTAATAAGCGGGGGCC-3′) and pDKWH1305 as the template to create pDKWH1305A. The mutated fragment was then cloned from pDKWH1305A into each of the 'A' mutant constructs described above to create a second set of *unc*I mutant derivatives of the fusion plasmids, designated 'B'. The correct constructions were verified by sequencing the resultant plasmids. The 'B' derivatives carry a 13 base pair deletion immediately after the first *Hin*dIII site in *unc*I followed by two stop codons and a frameshift of one base. Otherwise, the rest of the *unc*I DNA is intact. These 'B' derivatives therefore contain almost all of the *unc*I gene except that they do not code for a full-length i polypeptide. Instead they code for a peptide containing only 16 amino acids. Fusion genes were moved from the plasmids into λRZ5, and then into the chromosome using techniques described previously [9,21]. β-Galactosidase activities produced by these single-copy lysogens were assayed as described previously [21].

In vitro transcription-translation of purified λ DNA was carried out as described previously [22].

3. Results

3.1. Deleting uncI from unc-lac fusions

Fig. 1 shows the locations of the five *unc*B′-′*lac*Z translational fusions and the one *unc*E′-′*lac*Z fusion. These fusions contain increasing amounts of *unc*B, so the fusion proteins produced by each contain the N-terminus and increasing amounts of the a subunit fused to β-galactosidase. Each construction also contains the *unc* promoter and the *unc*I gene. We deleted *unc*I from each by removing the 199 base pair section between the two *Hin*dIII sites in *unc*I (Fig. 1), giving the resultant constructions the designation 'A'. We then measured the

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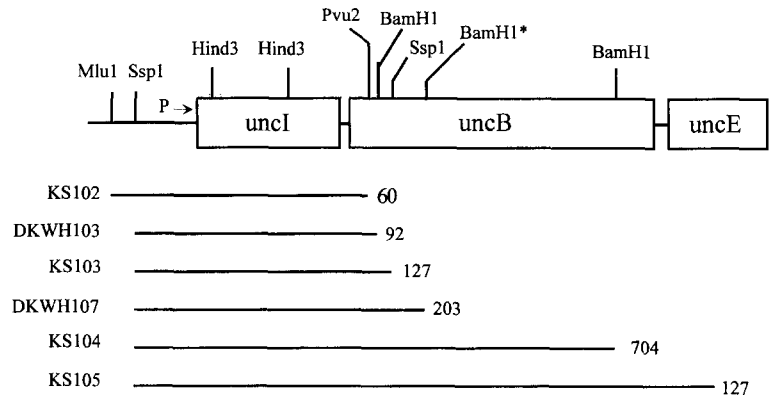

Fig. 1. Location of *lac* fusions. The first three genes of the *unc* operon are indicated with several of the restriction sites used for these constructions. The fusion constructions are listed on the left. The horizontal lines indicate the extent of *E. coli* DNA which has been fused in-frame to *lacZ* at a site indicated by the right-hand end of the line. The number following each line indicates how many bases of the *unc*B or *unc*E gene are present in each fusion. All fusions involve *unc*B except for KS105, which contains all of *unc*B and a fusion to *unc*E. The *Bam*H1* site is not normally present in *unc*B, but was constructed for the purpose of making the fusion in DKWH107.

effect of that deletion on the β-galactosidase activity produced from each of these fusions genes integrated into the chromosome in single copy. Table 1 summarizes the results. The *unc*I deletion had no effect on the expression of the KS102 fusion or the DKWH103 fusion, the two fusions containing the least amount of *unc*B or the a subunit. However, the activities produced by the other three fusion genes, KS103, DKWH107, and KS104, were all cut in half by the deletion.

The same deletion did not reduce the expression of an in-frame fusion to *unc*E, the gene following *unc*B. As shown in Table 1, the expression of a single-copy *unc*E′-′*lac*Z fusion gene in pKS105 actually increased by about a third when *unc*I was deleted.

As controls, we added the deleted section of *unc*I back to pKS104A and pKS105A, transfered each construction containing a now-intact *unc*I to λRZ5 and reintegrated the fusion genes into the chromosome. The activity produced by each fusion was restored to original levels, demonstrating that these constructions contained no additional mutations which affected their expression. The altered levels of expression observed in KS104A and KS105A were therefore caused by the *unc*I deletion.

3.2. *Time course of* β-*galactosidase activities produced by unc- lac fusions*

We measured the β-galactosidase produced by these fusions with and without the *unc*I deletion over a range of culture turbidities. The results are shown in Fig. 2. Over the entire range of culture turbidities measured, the *unc*I deletion had little or no effect on expression of the two early fusions to *unc*B, reduced the expression of the later fusions, and increased the expression of the fusion to *unc*E. The differences were therefore consistant and not growth-dependent in the OD range of 0.4 to 1.4.

3.3. *Effect of uncI deletion on in vitro synthesis of the a subunit*

We also measured the effects of this *unc*I deletion on the in vitro synthesis of the a subunit, using as a template purified λ DNA carrying the *unc* promoter followed by *unc*IBEF [23]. We constructed an identical λ missing the 199 base pairs of *unc*I, the same 'A' deletion described above for the *lac* fusion experiments. In vitro transcription-translation of purified λ DNA (Fig. 3) revealed the same result – the *unc*I deletion caused a significant reduction in the synthesis of the a subunit, with little or no effect on synthesis of the c subunit. The b subunit was not seen in the products of the DNA carrying the *unc*I deletion. The b subunit is very sensitive to the presence or absence of F_1 subunits when synthesized from this λ [23], so perhaps the decreased production of the a subunit makes b even more prone to proteolysis in the absence of F_1 subunits.

3.4. *Effects on unc-lac gene expression of uncI added in trans*

We attempted to reverse the effects of the *unc*I deletion by adding various plasmids containing *unc*I to each of these single-copy lysogens. We tested the effects of pWSB17 [13], pBS21 [15], and pBS81 [15], all of which have been shown to code for the synthesis of the *unc*I gene product. None of the *unc*I plasmids used had any effect on expression of the KS102, KS102A,

Table 1
β-Galactosidase activities produced by *unc*B′-′*lac*Z fusion genes in the presence and absence of *unc*I mutations

Strain	Activity ± S.E.M.		
	Control	I gene mutations	
		A	B
KS102	133 ± 4	138 ± 11	100 ± 1
DKWH103	115 ± 1	122 ± 5	97 ± 3
KS103	228 ± 3	112 ± 1	175 ± 1
DKWH107	103 ± 2	51 ± 1	100 ± 3
KS104	10 ± 1	4 ± 1	10 ± 1
KS105	142 ± 4	183 ± 2	125 ± 3

The three columns show the activities produced by single-copy fusion genes integrated into the λatt site in the *E. coli* chromosome. Column A shows the activities produced from each fusion if the 199 base pair *Hin*dIII fragment (see Fig. 1) is deleted from *unc*I. Column B shows the activities produced by these fusions if a 13 base pair deletion followed by two stop codons and a frameshift mutations is introduced immediately after the first *Hin*dIII site in *unc*I. Each number represents the average of activities measured from 8–10 different lysogens. The S.E.M. for each set of data is indicated.

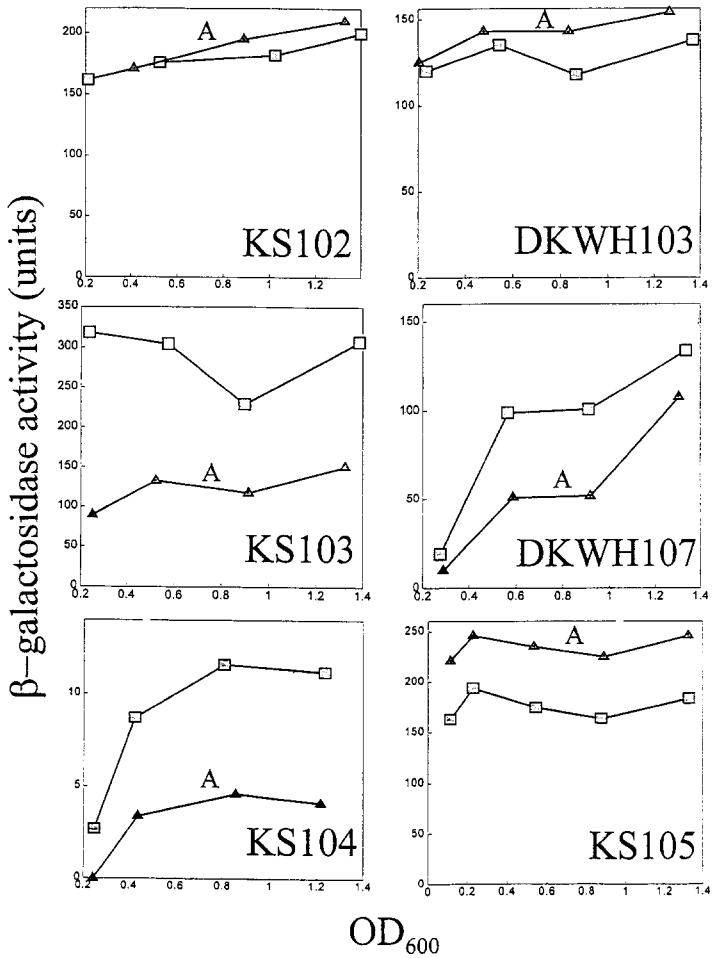

Fig. 2. Time course of β-galactosidase activities produced by single-copy fusion genes. The indicated λ lysogens were grown in minimal A medium [20] at 37°C. Samples were removed at the indicated culture turbitidies and assayed for β-galactosidase activity. Each panel shows the time-course of β-galactosidase activity present in cells carrying a lysogen (■) or the the *uncI* deletion (the 'A' construct) of that lysogen (△).

KS104, or DKWH104A fusions integrated in single copy in the chromosome (data not shown). The effect of the *uncI* gene on expression of *uncB* or synthesis of the a subunit was therefore not seen when the I gene was in *trans* to the B gene.

3.5. Effects on unc-lac expression of a second uncI mutation

We constructed a second mutation in the *uncI* gene carried by each of these fusion constructions. These mutations, designated 'B', contained almost all of the *uncI* gene, except that a small 13 base pair deletion together with stop codons and a frameshift were inserted near the first *Hin*dIII site in the gene. This construction was designed to determine whether the effects on *uncB* expression observed in the 'A' constructs were due to the *uncI* gene product or to the presence of *uncI* DNA or RNA. The 'B' mutation does not code for a protein, but does contain most of *uncI*. The activities produced by single copy 'B' constructs are shown in Table 1. The 'B' mutation had little effect on expression of any of the *uncB'-'lacZ* fusion genes. Overall expression of most of the genes dropped slightly, as if this mutation might be causing a slight decrease in the overall rate of transcription. These data indicate that the effects seen in the 'A' constructs were not caused by the absence of the *uncI* gene product but by the absence of *uncI* itself.

4. Discussion

The F_0 genes have been sequenced from at least 11 microorganisms [2–12]. In all of those, the gene for the a subunit is preceded by a mystery gene, designated I. This gene was first discovered in the sequencing of the *E. coli unc* operon [2]. The function of this gene, or the protein it codes for, remains unknown. Neither transposon mutagenesis of this region [18], nor a chromosomal deletion of *uncI* [14] had significant effects on measurable function of the ATPase. Yet the evolutionary conservation of this gene in this position within ATPase operons argues for some significant function of this gene in ATPase synthesis, assembly, or activity. Since cells are clearly capable of assembling functional ATPases in the absence of *uncI*, this gene or the protein it codes for would therefore have to serve some optimizing function. Some process involved in synthesis or assembly of the ATPase might occur more efficiently if the I gene is present, thus providing a slight but significant competitive advantage. The effect of the I gene could be too subtle to be detected by genetic and biochemical assays of ATPase function. In these experiments we have demonstrated that the I gene appears to be involved in expression of *uncB* or synthesis and assembly of the a subunit, or both.

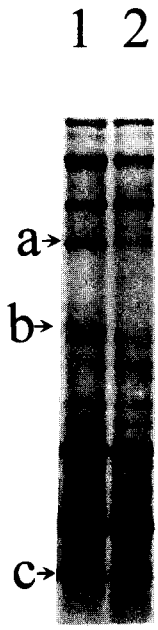

Fig. 3. In vitro transcription-translation of λF_0 DNA. In vitro transcription-translation was carried out in the presence of purified λ DNA. Proteins were labelled with [^{35}S]methionine, separated on a 13% polyacrylamide SDS gel, dried, and subjected to autoradiography. Lane 1 shows the proteins made from λF_0, and lane 2 shows the proteins made from an identical λF_0 carrying the *unc*I *Hin*dIII deletion. The locations of ATPase subunits are indicated alongside the figure.

The *unc*B gene codes for the a subunit, present in 1 copy per ATPase complex. This subunit is the most complex transmembrane subunit in the F_0, and several residues are believed to be involved in proton translocation across the membrane [1]. Yet the a subunit alone has been demonstrated to be harmful to growing cells. Overexpression of *unc*B has been shown to have deleterious effects on cell growth [24,25]. Additionally, studies on the *unc* transcript have shown that the mRNA transcript within the *unc*B cistron is more sensitive to cleavage and degradation than most of the genes in the operon [26–28]. The location of the gene for the a subunit is significant - despite coding for a single copy subunit, this gene precedes the most highly-expressed gene, *unc*E, which codes for the c subunit, present in 9 or more copies per complex. The intergenic region between *unc*B and *unc*E contains a translational enhancer which increases the number of ribosomes translating *unc*E [29]. It therefore appears as if the location, expression, and mRNA stability of *unc*B, the synthesis of the a subunit, and possibly the assembly of the a subunit into the membrane, is carefully controlled. Our data indicate that *unc*I is part of that control.

These studies do not directly address how *unc*I affects the synthesis or assembly of the a subunit. Clearly the effects are not due to a decrease in the overall transcription of the *unc* operon. The same mutation which decreases expression of three of the *unc*B'-'*lac*Z fusions has no effect on two other fusions and *increases* expression of the *unc*E'-'*lac*Z fusion gene. It is also unlikely that the *unc*I reading frame simply regulates the number of ribosomes presented to *unc*B in order to prevent overexpression and deleterious synthesis of the a subunit. The *unc*I deletion does not affect the activity produced by all the *unc*B'-'*lac*Z fusion genes, just those containing 127 or more base pairs of *unc*B. A more likely possibility is that the *unc*I mRNA is affecting the stability of the *unc*B mRNA. The *unc*B mRNA has been shown to be degraded more rapidly than most of the genes in the operon [26–28]. This mRNA degradation is believed to proceed from 5′ to 3′ and is catalyzed by RNaseE. The mRNA of *unc*I could be acting to delay the exposure of *unc*B to RNaseE, thus preventing premature inactivation of this cistron, which appears to be very sensitive to RNaseE-dependent degradation. Alternatively, the mRNA of *unc*I might base-pair with regions of the *unc*B mRNA and influence mRNA processing. For example, the 35 bp region of *unc*B between the first BamH1 site and the Sspl site contains a sequence of 6 C's. The *unc*I gene contains stretches of 4 and 5 G's between the *Hin*dIII sites, either one of which could base pair to the stretch of C's in *unc*B. In the absence of such putative base pairing, a variety of endonucleolytic sites in *unc*B could be made more or less acessible to RNase. Expression of the early fusions KS102 and DKWH103, which might not contain the sites for endonucleolytic attack, would then be unaffected by deleting *unc*I. In our view, some variation on this theme is the most likely explanation for the effects of *unc*I on synthesis of the a subunit.

This explanation does not explain the presence of an open reading frame in the mRNA preceding *unc*B. Either ribosomal binding and translation of *unc*I affects the availability of *unc*I mRNA for this *unc*B-protective function, or the *unc*I gene product has some additional function not obvious from these studies.

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