The Genes Encoding the Two Carboxyltransferase Subunits of *Escherichia coli* Acetyl-CoA Carboxylase*

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We report characterization of the component proteins and molecular cloning of the genes encoding the two subunits of the carboxyltransferase component of *Escherichia coli* acetyl-CoA carboxylase. Peptide mapping of the purified enzyme component indicates that the carboxyltransferase component is a complex of two nonidentical subunits, a 35-kDa α subunit and a 33-kDa β subunit. The α subunit gene encodes a protein of 319 residues and is located immediately downstream of the *polC* gene (min 4.3 of the *E. coli* genetic map). The deduced amino acid composition, molecular mass, and amino acid sequence match those determined for the purified α subunit. Six sequenced internal peptides also match the deduced sequence.

The amino-terminal sequence of the β subunit was found within a previously identified open reading frame of unknown function called *dedB* and *ug* (min 50 of the *E. coli* genetic map) which encodes a protein of 304 residues. Comparative peptide mapping also indicates that the *dedB*/*ug* gene encodes the β subunit. Moreover, the deduced molecular mass and amino acid composition of the *dedB*/*ug*-encoded protein closely match those determined for the β subunit. The deduced amino acid sequences of α and β subunits show marked sequence similarities to the COOH-terminal half and the NH₂-terminal halves, respectively, of the rat propionyl-CoA carboxylase, a biotin-dependent carboxylase that catalyzes a similar carboxyltransferase reaction. Several conserved regions which may function as CoA-binding sites are noted.

Acetyl-CoA carboxylase catalyzes the first committed step in fatty acid synthesis (Alberts and Vagelos, 1972). In *Escherichia coli* the enzyme is a complex that catalyzes two distinct half-reactions (Alberts and Vagelos, 1972; Guchhait et al., 1974). Biotin carboxylase catalyzes the carboxylation of protein bound biotin with bicarbonate followed by carboxyltransferase-catalyzed transfer of the carboxyl group carboxybiotin to acetyl-CoA to form malonyl-CoA (Scheme 1)(Alberts and Vagelos, 1972; Polakis et al., 1974). A third subunit, the biotin carboxyl carrier protein (BCCP), carries the biotin prosthetic group covalently attached to a lysine residue proximal to the carboxyl terminus (Sutton et al., 1977).

Although their reaction products are diverse, the reactions catalyzed by all biotin enzymes are thought to proceed by similar two-step mechanism (Samols et al., 1988; Knowles, 1989). All eukaryotic and most bacterial enzymes use bicarbonate and ATP to generate carboxybiotin, whereas carboxyl acceptors can be acyl-CoAs (e.g. as propionyl-CoA, acetyl-CoA, β-methyl-crotonyl-CoA, geranyl-CoA) or carboxylic acids such as pyruvate or urea (Daksihanurmi and Ghagavan, 1985; Wood and Barden, 1977). A second class of biotin enzymes serves as an energy transducer in anaerobic bacteria that couples decarboxylation to a sodium pump. The carboxyl donors are β-keto acids or their CoA derivatives such as oxalacetate, methylmalonyl-CoA, and glutacetyl-CoA (Dimroth, 1987). A third class is the *Propionibacterium shermanii* transcarboxylase which catalyzes a carboxyltransfer between carboxylic acids and acyl-CoAs that does not require ATP or CO₂ (Wood and Barden, 1977).

We have previously reported the isolation and characterization of the genes encoding the biotin carboxylase and biotin carboxyl carrier protein components of *E. coli* acetyl-CoA carboxylase (Li and Cronan, 1992). In this paper we report characterization of the α and β subunits of the carboxyltransferase component of this enzyme and the DNA sequence of the α subunit. We also demonstrate that the β subunit is encoded by the previously sequenced *dedB*/*ug* gene (Nonet et al., 1987; Bognar et al., 1987).

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Carboxyltransferase Purification and Characterization—Carboxyltransferase was purified to >95% purity by the method of Guchhait et al. (1974) except that fast protein liquid chromatography on a Mono Q column replaced the

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M96394.

1 The abbreviations used are: BCCP, biotin carboxyl carrier protein; NCS, N-chlorosuccinimide; SDS, sodium dodecyl sulfate; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; bp, base pair; kb, kilobase pair; HPLC, high performance liquid chromatography.

2 Portions of this paper (including "Experimental Procedures," Table 1, and Figs. 1, 2, and 4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
second DEAE column. A single protein peak eluted midway through the gradient (50 to 500 mM potassium phosphate) contained the carboxyltransferase activity. This protein fraction migrated with an apparent molecular mass of 130 kDa when analyzed by native gradient gel electrophoresis (an extension of the Ferguson method (Andrews, 1986)). Upon denaturing SDS-polyacrylamide electrophoresis, this fraction gave two protein bands of apparent molecular masses of 35 and 31.5 kDa that were stained with equal intensities by Coomassie Blue. The ratio of these two protein species was constant during the carboxyltransferase purification (Fig. 1) indicating that the carboxyltransferase consists of two subunits which we term $\alpha$ (35 kDa) and $\beta$ (31.5 kDa) in an $\alpha_2\beta_2$ complex. These observation agree well with those of Guchhait et al. (1974) who reported the E. coli carboxyltransferase to consist of 35- and 30-kDa subunits in a 2:2 complex.

The $\alpha$ and $\beta$ Subunits Are Encoded by Two Different Genes—The $\alpha$ and $\beta$ subunits could be related by post-translational modification (e.g. proteolysis) or be encoded by separate genes. Proteolysis seemed a distinct possibility since Guchhait et al. (1974) observed proteolysis of another protein (BCCP) isolated in the course of the carboxyltransferase purification. To examine this possibility, we performed comparative peptide mapping of the two subunits.

Peptide maps were obtained by two different means of protein fragmentation, trypsin digestion and cleavage with N-chlorosuccinimide (NCS), followed by separation of the peptides by either HPLC or SDS-gel electrophoresis. In both cases the peptide fragments of the $\alpha$ and $\beta$ subunits were clearly nonidentical, indicating that the proteins are encoded by two different genes. Moreover, we observed that the $\beta$ subunit was resistant to NCS treatment which cleaves at tryptophan residues. The apparent molecular weight was reduced only slightly by NCS treatment, suggesting that the $\beta$ subunit contains few tryptophan residues and that these must be located close to the ends of the protein chain.

Cloning of the $\alpha$ Subunit Gene—The NH$_2$-terminal sequence of the $\alpha$ subunit was obtained from the intact protein (Table 1) and this information was used to clone the encoding gene. An oligonucleotide probe of 32 bases was synthesized based on the least degenerate part of the amino-terminal sequence and the codon usage bias of moderately expressed E. coli genes. Four inosine residues were included at positions where no clear nucleotide choice could be made. The $^{32}$P end-labeled oligonucleotide was used as a hybridization probe in Southern blots of chromosomal DNA digested with various restriction enzymes. Specific signals were detected under highly stringent hybridization conditions. The predicted level of identity between the probe and the target sequences was >85% as calculated from the hybridization temperature and salt concentration. The low signal to noise ratio obtained from the above experiments encouraged us to screen the ordered miniset of the E. coli chromosomal bank of Kohara et al. (1987) immobilized on a membrane. Only two overlapping phage $\lambda$ clones, $\lambda$E2B8 and $\lambda$11D11, hybridized. These two recombinant phages carry a common chromosomal segment mapping at min 4.3 of the E. coli chromosome. A 7.2-kb BamHI fragment containing this common segment was subcloned from the phage $\lambda$E2B8 DNA and the hybridizing sequence was further localized by Southern blot analysis of restriction digests of the BamHI fragment. Nucleotide sequence analysis of a 700-bp Real subclone of the BamHI fragment gave a sequence that showed 90% identity with the probe sequence (omitting the 4 inosine residues). As expected, this sequence was found at the start of an open reading frame (ORF), and the beginning of the translated ORF sequence exactly matched the amino-terminal sequence, over 23 amino acid residues were determined for the $\alpha$ subunit (Table 1) except that the determined sequence lacked the initiating methionine (consistent with the known specificity of the NH$_2$-terminal protease (Hire, et al., 1989)).

The complete nucleotide sequence of the 960-bp ORF was determined from various subclones of the 7.2-kb BamHI fragment (Fig. 3). The calculated molecular weight (35,114 minus the NH$_2$-terminal methionine) of the ORF-encoded protein closely matched the 35-kDa measured molecular mass of the $\alpha$ subunit. The deduced and determined amino acid compositions also agreed well (Table 1). In addition to the amino-terminal sequence, the sequences of the four internal peptides and of the carboxyl-terminal peptide obtained by HPLC purification from cyanogen bromide or trypsin digests

![Figure 3](image-url)
Genes Encoding the Two Carboxyltransferase Subunits of \textit{E. coli}

16843

were found to match sequences of the translated ORF exactly (Fig. 3). Thus, this gene clearly encodes the \( \alpha \) subunit and we designate the gene \textit{accA}.

**Plasmid-directed Protein Synthesis**—The location, size, and transcriptional orientation of the putative \( \alpha \) subunit gene were further confirmed by analysis of maxicells and in vitro transcription-translation products. A \(^{35}\text{S}\)-labeled protein of 35 kDa was encoded by plasmids carrying either the 7.2-kb \textit{BamHI} insert or the 3.2-kb \textit{EcoRV} insert (Fig. 4). Truncated proteins of the sizes predicted from the DNA sequence were encoded by plasmids containing the 700-bp \textit{SstI} fragment and a series of deletions that retained the NH\(_2\) terminus of the protein. No labeled proteins other than the vector encoded proteins were detected in subclones that lacked the NH\(_2\)-terminal region (Fig. 4).

Our maxicell analyses indicated that another protein with an estimated molecular mass of 130 kDa was encoded by this 7.2-kb \textit{BamHI} fragment. This gene was mapped upstream of the \( \alpha \) subunit gene by in vitro translation of restriction fragments (Fig. 4). Truncated proteins of 68 and 40 kDa were seen with the upstream \textit{EcoRV} and \textit{HindIII} fragments, respectively, indicating that this gene was transcribed in the same direction as \textit{accA} (Fig. 4).

The 130-kDa protein encoded upstream of \textit{accA} is PolC, the catalytic subunit of DNA polymerase III (also called DnaE). This was established by (i) specific hybridization of the fragment to the phages of the Kohara library (Kohara \textit{et al.}, 1987) which carry the \textit{polC} \textit{E. coli} chromosomal segment, and (ii) perfect alignment of the DNA sequence downstream of the \textit{polC} gene reported by Tomaszewicz and McHenry (1987) with that encoding the amino-terminal sequence of \textit{accA} (Fig. 3). Correlation of our restriction map to that reported by Kohara \textit{et al.} (1987) indicates these two genes are located at 212.57 kb on the \textit{E. coli} physical map (near min 4.3 of the genetic map) and that both genes are transcribed in the clockwise direction (Médigue \textit{et al.}, 1991).

**Identification of the Gene Encoding the \( \beta \) Subunit**—The amino-terminal sequence of the isolated \( \beta \) subunit was obtained (Table 1) and a match to this sequence was found in the GenBank data base. The matched sequence was an ORF of unknown function called both \textit{dedB} (\textit{ded} denoting DNA downstream of \textit{hisT}) and \textit{usg} (for gene upstream of \textit{folC}) located between \textit{hisT} and \textit{purF} at min 50 of the \textit{E. coli} genetic map (Nonet \textit{et al.}, 1987; Bognar \textit{et al.}, 1987) (2440.97 kb of the physical map (Médigue \textit{et al.}, 1991)). The \textit{dedB}/\textit{usg} ORF was predicted to encode a protein of 33 kDa and a protein of this size was synthesized by maxicells carrying \textit{dedB}/\textit{usg} plasmids (Nonet \textit{et al.}, 1987; Bognar \textit{et al.}, 1989). Further evidence that the \textit{dedB}/\textit{usg} ORF encoded the \( \beta \) subunit came from the comparative peptide mapping. As expected from the resistance of the \( \beta \) subunit to NCS, the \textit{dedB}/\textit{usg} gene encoded a protein containing only 2 tryptophan residues, both located within the first 24 residues (Fig. 5). We also compared the peptide maps (NCS digestion) of the purified \( \beta \) subunit and the \(^{35}\text{S}\)methionine-labeled product of the \textit{dedB}/\textit{usg} gene (obtained by \textit{in vitro} transcription-translation) and found that the high molecular mass products co-migrated on SDS gels (Fig. 2). Digestion of the \( \beta \) subunit with cyanogen bromide, trypsin, and papain also gave products with molecular masses consistent with those predicted from the \textit{dedB}/\textit{usg} sequence (data not shown).

It should be noted that the molecular mass of the \( \beta \) subunit predicted from the \textit{dedB}/\textit{usg} DNA sequence (33,188 Da) (given removal of the NH\(_2\)-terminal methionine by aminopeptidase) is larger than (31.5 kDa) we observed for the purified \( \beta \) subunit (Fig. 1) and the 30-kDa value of Guchhait \textit{et al.} (1974). This discrepancy cannot be attributed to abnormal migration on SDS gels, since the protein encoded by the \textit{dedB}/\textit{usg} gene in maxicells (Nonet \textit{et al.}, 1987; Bognar \textit{et al.}, 1989), \textit{in vitro} (Fig. 2), or \textit{in vivo} (Fig. 1) migrates as a 33-kDa protein. The lower molecular weight of the isolated \( \beta \) subunit seems due to proteolysis during protein purification since the observed NH\(_2\)-terminal sequence (Table 1) began 15 residues downstream of the nearest initiation codon. Indeed, when the mass and composition of the deduced sequence were determined (31.7 kDa) and (31.7 kDa) molecular masses and amino acid compositions (Table 1) agreed well. Proteolytic clipping also explained the resistance of the isolated \( \beta \) subunit to NCS digestion. The isolated 31.5-kDa subunit would contain only a single tryptophan residue and NCS cleavage would remove only 8 residues from the protein (Fig. 5). It should be noted that prior maxicell analysis of various deletions within the \textit{dedB}/\textit{usg} sequence indicates that the initiation methionine is that shown in Fig. 5 rather than an upstream codon (Bognar \textit{et al.}, 1989).

**Discussion**

We have shown that the carboxyltransferase component of \textit{E. coli} acetyl-CoA carboxylase consists of two nonidentical protein species, a 35-kDa \( \alpha \) subunit and a 33-kDa \( \beta \) subunit. We report cloning, sequencing, and mapping of the gene encoding the \( \alpha \) subunit and have demonstrated that the \( \beta \) subunit is encoded by the previously sequenced \textit{dedB}/\textit{usg} gene. We propose that the \textit{dedB}/\textit{usg} gene designation be renamed \textit{accD}. The designations of \textit{accA}, \textit{accB}, and \textit{accC}, have been assigned to genes encoding the carboxyltransferase \( \alpha \) subunit, BCCP, and biotin carboxylase, respectively. The sequences of the \( \alpha \) and \( \beta \) subunits were searched versus the GenBank data base and the only proteins showing significant similarities were known biotin carboxylase enzymes. Both carboxyltransferase subunits showed strong similarities to the \( \beta \) subunit of rat propionyl-CoA carboxylase. Unlike other eukaryotic biotin-dependent carboxylases (Knowles, 1989), the functional form of rat propionyl-CoA carboxylase is a complex of \( \alpha \) and \( \beta \) subunits (Samols \textit{et al.}, 1988). The biotin carboxylase activity and biotinylated domain was thought to reside in the \( \alpha \) subunit, whereas the \( \beta \) subunit was thought to catalyze the carboxyltransferase reaction (Samols \textit{et al.}, 1988). We have previously shown that the biotin carboxylase and BCCP subunits of \textit{E. coli} acetyl-CoA carboxylase have many sequences in common with the \( \alpha \) subunit of rat propionyl-CoA carboxylase (Li and Cronan, 1992). The alignment of the \textit{accA} and \textit{accD} amino acid sequences to the \( \beta \) subunit of this enzyme is consistent with this functional assignment. Indeed, the \textit{E. coli} carboxyltransferase \( \beta \) subunit is readily aligned with the amino-terminal half of the rat \( \beta \) subunit, whereas the \textit{E. coli} \( \alpha \) subunit can be aligned with the carboxyl-terminal half (Fig. 5).

AccA and AccD also show sequence similarity to the 12 S subunit of \textit{P. shermanii} transcarboxylase (Fig. 6). The transcarboxylase 12 S subunit of \textit{P. shermanii} and the rat propionyl-CoA carboxylase \( \beta \) subunit both catalyze carboxyltransfer from carboxybiotin to propionyl-CoA, a reaction mechanistically similar to the half-reaction catalyzed by the \textit{E. coli} carboxyltransferase (Wood and Barden, 1977; Knowles, 1989). Both the 12 S transcarboxylase subunit and propionyl-CoA carboxylase \( \beta \) subunit contain putative acyl-CoA-binding sites (Samols \textit{et al.}, 1988) and these are conserved in the \textit{E. coli} protein. Moreover, the \textit{E. coli} carboxyltransferase has been shown to bind acetyl-CoA and malonyl-CoA (Alberts \textit{et al.}, 1971). As shown in Fig. 6, the most conserved regions
Genes Encoding the Two Carboxyltransferase Subunits of E. coli

FIG. 5. Alignments of the entire E. coli carboxyltransferase α (AccA) and β (AccD) subunit sequences with the sequence of rat propionyl-CoA carboxylase β subunit (RPCCB; Kraus et al., 1986). Identical residues are boxed. The site of the proteolytic cleavage believed to occur during purification (see text and Table 1) is marked with a vertical arrow.

ACCA (84–119)  RPCCB (327–360)  PS12S (298–331)  LACA (133–150)  Acetyl-CoA
RPP12 (298–331)  ACCD (116–151)  RPCCB (117–152)  ACCE (152–170)  Carbamoyl-phosphate

ACCD (152–170)  RPCCB (153–171)  RS12 (128–146)  LACA (1708–1726)  GAMMA-glutamyltransferase

ACCD (152–170)  RPCCB (153–171)  RS12 (128–146)  LACA (1708–1726)  GAMMA-glutamyltransferase

among these enzymes are those between residues 110 and 220 of ACCD and residues 80 and 190 of ACCA. When compared to enzymes known to utilize acetyl-CoA, the sequence found between residues 84 and 119 of ACCA was found remarkably similar to those of rat acetyl-coA carboxylase (residues 1955–1988) and E. coli thiogalactoside acetyltransferase (LacA; residues 133–150) (Fig. 6). These sequences are those previously proposed (Samols et al., 1988; Lopez-Casillas et al., 1988) to be acyl-CoA-binding domains (Fig. 6A). Moreover, the putative acyl-CoA binding sequences conserved among the biotin-dependent carboxylases contains a periodically repeated sequence similar to the predicted hexapeptide repeat motif found in LacA and eight other acetyltransferases (Vuorio et al., 1991). Conservation of a repeated sequence rather than of a defined sequence may reflect the mechanistic differences of these two enzyme families. The biotin-dependent carboxylases utilize acyl-CoAs as carboxyl acceptors, whereas acetyltransferases transfer the acetyl group to an acceptor molecule (Vuorio et al., 1991). These considerations suggest that the CoA-binding site of the E. coli carboxyltransferase resides in the α subunit. The lack of strong similarities between rat propionyl-CoA carboxylase and the amino-terminal third of the α subunit (Fig. 5) might be due to the differing substrate specificities of the two enzymes. The E. coli enzyme is an unusual acetyl-CoA carboxylase in that it fails to carboxylate propionyl-CoA, but carboxylates free biotin (Alberts and Vagelos, 1968; Knowles, 1989).

Both the rat propionyl-CoA carboxylase β subunit and E. coli carboxyltransferase interact with carboxybiotin and catalyze carboxyltransfer (Kraus et al., 1986). Thus, these enzymes should have specific binding sites for carboxybiotin. Sequence similarities found between residues 116 and 170 of the E. coli carboxyltransferase β subunit and residues 117 and 171 of the rat propionyl-CoA carboxylase might represent carboxybiotin-binding sites (Fig. 6B). Similar conserved regions can also be found in the sequences of two other biotin enzymes catalyzing analogous reactions, the rat acetyl-CoA carboxylase (RACC; Lopez-Casillas et al., 1988), and E. coli thiolgalactoside acetyltransferase (LACA; Vuorio et al., 1991). Numbers in parentheses indicate the amino acid positions of each protein. Residues identical in three sequences are boxed. The periodicity of the proposed hexapeptide motif (Vuorio et al., 1991) is marked beneath the LacA sequence.

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regions in other biotin enzymes were unsuccessful, perhaps because most biotin enzymes require protein-bound biotin for the carboxylation and carboxyltransfer activities (the only enzymes reported to act on free biotin are E. coli acetyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase) (Wood and Barden, 1977). Note, that even the two catalytic components of E. coli acetyl-CoA carboxylase differ in their specificity towards the valerate side chain of the biotin molecule (Polakis et al., 1974). Therefore, detection of the sequences responsible for binding of carboxybiotin seems difficult and may be further complicated by the differing substrate-binding sites of biotin enzymes. Our assignments of the putative carboxybiotin-binding region to the β subunit and the putative CoA-binding domain to the α subunit are consistent with the catalytic properties of the carboxyltransferase component. Carboxyltransferase catalyzes a slow biotin-independent decarboxylation of malonyl-CoA (Guchhait et al., 1974). 2-Imidazolidione, an analogue of the ureido ring of biotin, does not accept the CO₂ group, but accelerates malonyl-CoA decarboxylation (Polakis, et al., 1974), suggesting that the binding sites for biotin and malonyl-CoA are physically distinct and could reside on different subunits.

The accD gene (mapped at min 50 of the E. coli chromosome) is not closely linked either to the BCCP and biotin carboxyltransferase genes (mapped at min 72) or to the gene encoding the carboxyltransferase α subunit (mapped at min 4.3). Carboxyltransferase, BCCP, and biotin carboxylase function as a complex, presumably of defined stoichiometry (Guchhait et al., 1974; Alberts and Vagelos, 1972). We have previously shown that BCCP and biotin carboxylase genes are co-transcribed (Li and Cronan, 1992). Hence, a thorough study of this operon together with the accD and accA genes may explain how E. coli coordinates the expression of these genes to form a functional acetyl-CoA carboxylase.

Finally, our finding that the AccA and AccB proteins both show significant amino acid sequence similarities to the rat propionyl-CoA carboxylase β subunit suggests that the rat β subunit gene arose by fusion of accA- and accB-like genes. However, a second possibility is that the separation of the accA and accB sequences resulted during the evolution of the E. coli chromosome. Riley and Annilionis (1978) have proposed that the present E. coli chromosome arose by two successive duplications of a smaller chromosome resulting in genes being displaced by either one-fourth or one-half of the genome. Since, the accA and accD genes are located almost precisely opposite one another on the map (at min 4 and 50, respectively), it seems possible that the primordial carboxyltransferase may have consisted of a single protein that became split during chromosome evolution.

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Genes Encoding the Two Carboxyltransferase Subunits of E. coli

Experimental Procedures

Genes Encoding the Two Carboxyltransferase Subunits of E. coli

Supplemental Material

L. D. Bionu and J. E. Cronan, Jr.

Fig. 1. Carboxyltransferase Purification. Lanes 1, 2, and 3 are molecular mass standards; lane 2, crude cell extract; lane 3, ammonium sulfate precipitate; lane 4, pooled active hydroxypropyl eluates; lane 5, DEAE cellulose chromatography; lane 6, chromatography; lane 7, pooled active fractions from the Mono Q column. Lane 9, crude extract of a strain carrying the α subunit overproduction plasmid; lane 10, as in lane 9 except the plasmid encoded the β subunit; lane 11, strand lacking plasmid; lane 12, purified α and β subunits.

Fig. 2. Comparative Peptide Mapping of the Carboxyltransferase Subunits. A, NCIC profiles of peptides obtained by tryptic digestion of the α (upper panel) or β (lower panel) subunits. B, SDS-PAGE analysis of NCIC-digested subunits. Lane 1, untreated α subunit; lane 2, NCIC-treated α subunit; lane 3, untreated β subunit; lane 4, NCIC-treated β subunit; lane 5, untreated α subunit; lane 6, untreated β subunit. C, SDS-PAGE analysis of the products of in vitro transcription/translation of a plasmid encoding the β subunit gene. Lane 3 is the untreated product and lane 4 is the NCIC-treated product.
Fig. 4. DNA-directed protein synthesis. Panel A: Restriction map of the 7.1 kb BamHI fragment of Khorana phage clone 132/846. The restriction fragments used in construction of plasmids for masscell analysis or in vitro transcription/translation are shown in bold letters beneath the restriction map. Panel B: Proteins encoded by masscell containing various plasmids. Lanes 1 and 16, the vector plasmid p8188; lane 2, plasmid p8197 (which carries the left BamHI-DraI fragment); lane 3, plasmid p8198 (which carries the left EcoRV-BamHI fragment); and lane 4, plasmid p8199 (which carries the right BamHI-DraI fragment). Lanes 8 and 7 are in vitro transcription/translation products of linearized plasmids (carrying the left BamHI-DraI fragment) laned linear in panel A); and the left BamHI-DraI fragment). Lanes 8-13 are the proteins encoded in masscells with various plasmids. Lane 9, p8198 (which carries the right EcoRV-BamHI fragment); lane 11, plasmid p8199 (which carries the right BamHI-DraI fragment). Lanes 13, plasmid p8119 (which carries the larger BamHI-AamI fragment); lane 14, plasmid p8119 (which carries the Clal-HindIII fragment); and lane 15, plasmid p8119 (which carries the right BamHI-DraI fragment). The positions of β-lactamase, pre-β-lactamase, and of the intact and truncated derivatives of PsIC and AcmC are marked. 

Table 1. Determined amino terminal sequences and amino acid compositions of the carboxyltransferase subunits

| Amino terminal sequences* | B subunit | C subunit |
|---------------------------|-----------|-----------|
| cycle | residue | yield (mol/mol) | residue | yield (mol/mol) |
| 1 | L | 21.1 | A | 29.9 |
| 2 | N | 11.7 | S | 18.7 |
| 3 | L | 20.9 | P | 12.3 |
| 4 | D | 12.3 | E | 14.1 |
| 5 | F | 9.0 | G | 10.2 |
| 6 | E | 10.1 | V | 1.4 |
| 7 | O | 4.9 | V | 4.7 |
| 8 | A | 8.3 | P | 7.3 |
| 9 | R | 5.7 | G | 9.5 |
| 10 | L | 6.9 | S | 4.4 |
| 11 | T | 8.6 | | |
| 12 | K | 2.3 | | |
| 13 | I | 1.8 | | |
| 14 | D | 1.0 | | |
| 15 | G | 4.6 | | |
| 16 | A | 4.7 | | |
| 17 | V | 2.4 | | |

(a) Values obtained by automatic Edman sequencing of protein bands resolved by SDS-PAGE. Cleavage of the N-terminal methionine of a subunit is consistent with the in vivo specificity of the amino peptidase (Kier et al., 1988). The question mark denotes cycles in which no released residue was observed.

(b) Values calculated by subtracting the composition of the first fifteen amino terminal residues from the amino acid composition deduced from the DNA sequence (Fig. 3).

(c) Values deduced from the sequence shown in Fig. 3.

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Genes Encoding the Two Carboxyltransferase Subunits of E. coli

16847