Translational initiation frequency of atp genes from
*Escherichia coli*: identification of an intercistronic
sequence that enhances translation

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The c, b and δ subunit genes of the *Escherichia coli* atp operon
were cloned individually in an expression vector between the
tac fusion promoter and the gatK gene. The relative rates of
subunit synthesis directed by the cloned genes were similar
*in vitro* and *in vivo* and compared favourably with the subunit
stoichiometry of the assembled proton-translocating ATP
synthase of *E. coli* *in vivo*. The rate of synthesis of subunit c was
at least six times that of subunit b and 18 times that of subunit δ.
Progressive shortening of the long intercistronic sequence
lying upstream of the subunit c gene showed that maximal
expression of this gene is dependent upon the presence of a
sequence stretching > 20 bp upstream of the Shine-Dalgarno
site. This sequence thus acts to enhance the rate of translational
initiation. The possibility that similar sequences might
perform the same function in other operons of *E. coli* and
bacteriophage λ is also discussed. Translation of the subunit
b cistron is partially coupled to translation of the preceding
subunit c cistron. In conclusion, the expression of all the atp
operon genes could be adjusted to accommodate the subunit
requirements of ATP synthase assembly primarily by means
of mechanisms which control the efficiency of translational
initiation and re-initiation at the respective cistron start
codons.

Key words: *E. coli* atp operon/subunit stoichiometry/*in vitro* and
*vivo* expression/translational initiation

Introduction

The proton-translocating ATP-synthase (H⁺-ATPase) of
*Escherichia coli* is composed of eight different types of subunit,
the complete genetic information for which lies in a single
operon (designated unc or atp; see Figure 1) at ~ 83 minutes on
the *E. coli* linkage map (Downie et al., 1979; Friedl et al.,
1979; Futai and Kanazawa, 1983; Gay and Walker, 1981a,
1981b; Saraste et al., 1981; Nielsen et al., 1981). The F₁
part of the enzyme, which alone has ATP hydrolyase activity,
comprises five types of subunit (α, β, γ, δ and ε), whereas
the membrane-integrated component F₀ comprises three types
of subunit (a, b and c; see, e.g., Hoppe and Sebald, 1984).
DNA sequencing (Gay and Walker, 1981b) has revealed the
existence of a ninth, apparently non-essential (von Meyenburg
et al., 1982a) gene (designated gene 1 or ap1) at the
beginning of the atp operon (see also Brusilow et al., 1983).

A particularly remarkable aspect of not only the structure
of the *E. coli* H⁺-ATPase, but also of the mitochondrial and
thylakoid H⁺-ATPases, is the stoichiometry of the compo-
ment subunits. The relative molar quantities of the *E. coli*
subunits estimated on the basis of radioactivity incorporation
studies are α3β2γ2δε1.5 (Foster and Fillingame, 1982;
von Meyenburg et al., 1982b; see also Lünsdorf et al., 1984).
How is this stoichiometry achieved in the synthesis and
assembly of the *E. coli* H⁺-ATPase, or indeed of other
H⁺-ATPases?

Several lines of evidence, including the results of S1 nuclease
mapping, indicate that the atp operon has a single major pro-
moter which initiates transcription 73 bp upstream from the
gene 1 (ap1) reading frame (von Meyenburg et al., 1982a;
Porter et al., 1983; Jones et al., 1983; Nielsen et al., 1984;
Kanazawa et al., 1981). Thus the atp operon is transcribed
to produce a single, large mRNA species containing all of
the structural gene reading frames. One hypothesis to explain
the attainment of the subunit stoichiometry of the *E. coli*
H⁺-ATPase has accordingly been that the rates of synthesis
in the respective subunits are regulated at the level of poly-
peptide chain elongation (see, e.g., Futai and Kanazawa,
1983). On the other hand, Brusilow et al. (1982) proposed
that mRNA secondary structure might play an important role
in suppressing translational initiation at certain sites (correspon-
ding to the starts of proteins b, δ and γ).

The present work demonstrates that differences in the
efficiency of translational initiation at different sites in the *E.
coli* atp operon polycistronic message play a major role in
determining the relative amounts of the different subunits that
are synthesized. In particular, a specific mechanism of
enhancement of translational initiation in the case of the subunit
c cistron is shown to be important for the attainment of the
required ratio of synthesized subunits. A preliminary report
of some of the results reported in this paper has already been
given (McCarthy et al., 1984).

Results

Cloning and expression of the genes for H⁺-ATPase subunits
b, c and δ

The genes coding for subunits c, b and δ from the atp operon

![Diagram](https://example.com/diagram.png)

Fig. 1. The structure and a partial restriction map of the atp operon.
The diagram shows the nine genes of the operon together with the
major promoter (P) and terminator (T). Restriction endonuclease sites
which were useful for the construction of the described plasmids are
*Taq*I. Data obtained from sources given in text.
J.E.G. McCarthy, H.U. Schairer and W. Sebald

Fig. 2. The structure of a subunit c gene expression plasmid. A typical plasmid is shown (see lane 7, Figure 3). pH116(c7) was constructed by inserting a fragment of the atp operon bearing the subunit c gene into the BamHI site of pH184. The long arrows indicate the direction of the common transcript. The small arrows indicate the sites of five translation stop codons.

(atpE, atpF and atpH, respectively) were cloned using the plasmid pDR540 (Russell and Bennett, 1982; see Materials and methods). Fragments of the operon were inserted into the BamHI site of the vector so that transcription of either the individual genes of the b, c and δ subunits, or the b and c subunit genes together, was initiated by the tac promoter and transcription continued through the galK gene. A diagram of the structure of one of the recombinant plasmids bearing just the c subunit gene is shown in Figure 2. Expression of the galK gene (see, e.g., McKenney et al., 1981) served as a useful reference indicator of the activity of the tac promoter. The 35S-labelled products of in vitro protein synthesis directed by each of the plasmids were readily identified (see Materials and methods and Figure 3). These proteins ran at precisely their expected positions on the gels. In the fluorograph depicted in Figure 3 the bands corresponding to β-lactamase and galactokinase, whose synthesis is directed by their expected positions on the gels.

Comparison of the expression of the different plasmid-borne genes

The relative amounts of synthesis of subunits c, b and δ of galactokinase directed in vitro by the constructed plasmids were measured over a wide range of plasmid DNA concentrations. Thus in Figure 5A the relative amounts of subunits c and b and of galactokinase separated by SDS-polyacrylamide electrophoresis have been plotted as a function of the concentration of pB1050(cb8). pB1050(cb8) bears the genes of both subunits c and b together with the complete intercistronic sequence upstream of the subunit c gene and 19 bp of the subunit δ gene. The background radioactivity in the gels run with in vitro protein synthesis products was more significant in the case of subunit b than of subunit δ because subunit b ran close to an undefined polypeptide of the in vitro system. The background radioactivity in the regions of subunit c and galactokinase was negligible in relation to the incorporation into these proteins. Correcting for background radioactivity as described in Materials and methods and also for the methionine content of the respective subunits allowed the calculation of a synthesis ratio of moles subunit c : moles subunit b of between 6 and 10. The lowest ratios were generally obtained at the lower concentration of DNA, a maximum was reached at a DNA concentration in the range 30 - 70 µg/ml, and a value of ~ 8 was calculated at the very highest DNA concentrations (see Figure 5A and Table I).

The expression of pB1050(cb10), which is identical to pB1050(cb8) except that it lacks most of the intercistronic sequence upstream of the subunit c gene, showed different characteristics. The synthesis of subunit c directed by this plasmid was extremely low (i.e., equivalent to that obtained with pH163(c3)) compared with the expression obtained with pB1050(cb8) (compare, e.g., lanes 8 and 10, Figure 3), and when the intercistronic sequence lying upstream of the Shine-Dalgarno sequence was progressively shortened (compare Figures 3 and 4 and Table I). The expression of the subunit c gene (in Table I normalized to the expression of galK) was equally high with all the plasmids bearing the complete intercistronic sequence upstream of this gene [pH184(c6), pH116(c7), pB10501(cb8) and pB10505(cb9); Figure 3; compare also Figure 4]. The ratio of subunit c:galactokinase obtained in vitro at a given DNA concentration with pH159(c5) and pH160(cb4) was respectively 20% and 3% of that determined with pH184(c6) (Table I). The effects of shortening the intercistronic sequence upstream of the subunit c gene upon the synthesis of subunit c in vitro could be quantitatively confirmed in vivo (see, e.g., Figure 4) within the limits of accuracy of the methodology applied (see Materials and methods). The synthesis of subunit c directed by the two plasmids with the shortest inserts [pH152(c2) and pH163(c3), Figure 3] was barely distinguishable from background (and not at all discernible in Figure 3), but it was shown in both cases by complementation (see Materials and methods) that functional subunit c could be produced in vivo.

Relatively the same effects of shortening the intercistronic sequence upstream of the subunit c gene upon the expression of this gene in vitro and in vivo were observed when the cloned fragment of plasmids pH116(c7), pH159(c5) and pH163(c3) were inserted behind the λP1 promoter in pLl101 (see Materials and methods). The products synthesized in vitro under the direction of one of these recombinant plasmids are shown in Figure 3 (lane 13).

Shortening the intercistronic sequence upstream of the subunit c gene reduces the synthesis of subunit c

The synthesis of the products shown in lanes 2 - 7 of Figure 3 was directed by a set of plasmids bearing the subunit c structural gene together with varying lengths of the intercistronic sequence upstream of it. The 3' ends of the cloned DNA fragments in these plasmids were all at the HpaI site between the genes encoding subunits b and c (see Figure 1); the 5' ends are defined precisely in Figure 3. Expression of the subunit c gene in vitro and in vivo was drastically reduced
Expression of genes from the E. coli atp operon

was thus consistent with the data presented in the previous section. Moreover, the subunit b gene was also relatively more weakly expressed by pB10506(cb10). Indeed, an equally weak expression of the subunit b gene to that obtained with pB10506(cb10) was observed with a plasmid [pHB6(b11)] which bears only the subunit b gene. The fragment cloned in pHB6(b11) was derived from pB10501(cb8) and begins 22 bp upstream of the subunit b gene start codon. Comparison of the radioactive incorporation ratios of subunit b:galactokinase for pHB6(b11) and pB10506(cb10) on the one hand, and pB10501(cb8) on the other (Table I), reveals a large difference, although this is partially attributable to the apparent competitive inhibitory effect of subunit c synthesis on galK expression. The number of pmol of subunit b synthesis directed per mg plasmid DNA was approximately two times less for pH6(b11) than for pB10501(cb8) over the DNA concentration range 5 - 200 mg/ml. A similar relationship for the expression of the subunit b gene as carried by these two plasmids was observed in vivo (Figure 4).

The in vitro synthesis products of a further b and c subunit plasmid [pB10505(cb9)] are shown in Figure 3. It is remarkable that although the cloned fragment of pB10505(cb9) had lost the last 12 bp of its subunit b gene sequence, pB10505(cb9) still complemented atp b− mutants (see

Fig. 3. In vitro expression of the genes of subunits c, b and δ from the atp operon. Fragments from the atp operon were cloned in the BamH I site of pDR540 (see Figure 2) or the Sali site of pJLF161 (see Materials and methods). The [35S]L-methionine-labelled proteins synthesized in the S30 system under the direction of these plasmids were separated on a 13.5% polyacrylamide gel which was subsequently dried and fluorographed. The plasmids used (all at 56 mg/ml) were pDR540 (lane 1); subunit c (atpE) plasmids pHI52 (lane 2), pHI63 (lane 3), pH106 (lane 4), pH159 (lane 5), pH184 (lane 6), pH116 (lane 7), pJLF161 (lane 13); subunits c−b (atpE and atpF) plasmids pB10501, pB10505 and pB10506 (lanes 8 – 10, respectively); subunit b plasmid pHB6 (lane 11); subunit δ plasmid pSE1 (lane 12). In the text the cloned genes and corresponding lane numbers of this figure are indicated in parentheses after the plasmid code: e.g., pB10501(cb8). A plasmid-free water blank experiment was also performed (lane 14). In each case 4% of the total counts were loaded onto the gel (see Materials and methods). Also shown in the lower part of the figure is the sequence of the end of the subunit a gene followed by the whole intercistronic sequence up to and including the start codon of the subunit c gene. The lines indicate the fragments of the operon cloned into pDR540 and are numbered according to the lane order of the gel. pJLF161 contains the same insert as pH116. s.c. = stop codon.
J.E.G. McCarthy, H.U. Schairer and W. Sebald

522

over a wide range of plasmid DNA concentrations (Figure 5B and Table I). The ratios of subunit b:galactokinase and subunit c:galactokinase obtained over a wide range of DNA concentrations with these two plasmids indicate, as do the specific in vitro yields of subunit per μg of each plasmid, that the expression efficiency is 1.5–2 times greater for the subunit b gene in pH6(b 11) than for the subunit δ gene in pSE1(δ12). Synthesis of the δ subunit directed by pSE1(δ12) in vivo was so low in relation to the synthesis of the c and b subunits (and to background) as to be barely measurable (Figure 4).

In vivo pulse-labelling of proteins encoded by the described recombinant plasmids. Pulse-labelling was performed as described in Materials and methods using strains carrying recombinant plasmids. The host strain was MCG1, which is apt - . A fluorograph was prepared from a 15% SDS-polyacrylamide gel which had been loaded with the sonicated and solubilized cells after pulse-labelling. Experiments were performed with MCG1 itself (lane 1), or with MCG1 transformed with pH163 (lane 2), pH159 (lane 3), pH116 (lane 4), pBI0501 (lane 5), pH6 (lane 6) or pSE1 (lane 7) (compare Figure 3). The letters on the right hand side indicate the positions of galactokinase (g), β-lactamase (l), the δ subunit, b subunit and c subunit, respectively.

Materials and methods). It is unclear why the loss of the last 12 bp of the subunit b gene results in the synthesis of a polypeptide with such a noticeably increased mobility on SDS-polyacrylamide gels.

The cloned fragment of pSE1(δ12) stretches from 84 bp upstream of the subunit δ gene start codon to 25 bp after this gene’s stop codon. A low incorporated radioactivity ratio (subunit δ:galactokinase from 0.091 to 0.063) was maintained over a wide range of plasmid DNA concentrations (Figure 5B and Table I). Expression of the subunit δ gene in pSE1(δ12) or of the subunit b gene in pH6(b 11) exerted no measurable effect upon expression of galK (relative to pDRS40). Thus the ratios of subunit δ:galactokinase and subunit b:galac-

Fig. 4. In vivo pulse-labelling of proteins encoded by the described recombinant plasmids. Pulse-labelling was performed as described in Materials and methods using strains carrying recombinant plasmids. The host strain was MCG1, which is apt - . A fluorograph was prepared from a 15% SDS-polyacrylamide gel which had been loaded with the sonicated and solubilized cells after pulse-labelling. Experiments were performed with MCG1 itself (lane 1), or with MCG1 transformed with pH163 (lane 2), pH159 (lane 3), pH116 (lane 4), pBI0501 (lane 5), pH6 (lane 6) or pSE1 (lane 7) (compare Figure 3). The letters on the right hand side indicate the positions of galactokinase (g), β-lactamase (l), the δ subunit, b subunit and c subunit, respectively.

Table I. Comparison of the synthesis of E. coli H+-ATPase subunits and galactokinase in the in vitro system

<table>
<thead>
<tr>
<th>Gene products analyzed</th>
<th>Plasmid</th>
<th>Ratio of [35S]L-methionine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A subunit c : subunit b</td>
<td>pBI0501(cb8)</td>
<td>10</td>
</tr>
<tr>
<td>B subunit c : galactokinase</td>
<td>pBI0501(cb8)</td>
<td>6.7</td>
</tr>
<tr>
<td>subunit b : galactokinase</td>
<td>pBI0501(cb8)</td>
<td>0.66</td>
</tr>
<tr>
<td>subunit g : galactokinase</td>
<td>pH6(b11)</td>
<td>0.16</td>
</tr>
<tr>
<td>C subunit c : galactokinase</td>
<td>pH184(c6)</td>
<td>7.6</td>
</tr>
<tr>
<td>subunit c : galactokinase</td>
<td>pH159(c5)</td>
<td>1.5</td>
</tr>
<tr>
<td>subunit c : galactokinase</td>
<td>pH100(c4)</td>
<td>0.24</td>
</tr>
<tr>
<td>subunit c : galactokinase</td>
<td>pH163(c3)</td>
<td>0.024</td>
</tr>
<tr>
<td>D subunit δ : galactokinase</td>
<td>pSE1(δ12)</td>
<td>0.091</td>
</tr>
</tbody>
</table>

* In each case the data given apply to a plasmid concentration of 69 μg/ml.
* These are molar stoichiometries calculated after correction for the content of methionines in each of the proteins [see references regarding the apn operon in the text and Wilson and Hogness (1969)] and also, in the cases of the b and δ subunits, for background (see Materials and methods).

Discussion
We have shown that the cloning of apn genes in expression vectors allows a detailed examination of their expression.
expression of genes from the E. coli ap operon

Fig. 5. Incorporation of [35S]methionine into specific proteins in the S10 in vitro system. Typical plots of the incorporation into specific protein bands as a function of the concentration of added plasmid DNA (pBI050l(c8h), A; pSEI(d12), B) are shown. The radioactivity of bands corresponding to galactokinase (- ○ -), subunit c (- △ -), subunit b (- ● -) and subunit δ (- △ -) was determined and divided by the number of methionines contained in each type of protein molecule (see left hand abscissae). Thus for each of the experiments A and B the plotted values represent the relative molar amounts of each protein synthesized. Also shown are the total amounts of [35S]methionine incorporated into protein (measured as hot trichloroacetic acid-insoluble radioactive material corrected for the radioactivity of water blanks; - - ● -). Different specific activities of [35S]-methionine were used respectively in A and B.

behaviour in vitro and in vivo. The c, b and δ subunits were stable and it was therefore possible to quantify reliably the large differences in the respective synthesis rates of these polypeptides. pDR540 was especially useful for quantitative analysis of the expression of the c, b and δ subunit genes because it has a built-in reference gene (galK) lying behind the cloning site which is transcribed as part of a common transcript with each of the cloned DNA sequences. Thus the measurement of the expression of galK together with ap genes cloned in this plasmid allowed the identification of differences in the efficiency of translation of these genes. Moreover, the relative levels of expression of the cloned ap genes in vitro and in vivo were also generally consistent with each other.

The differences in the respective rates of synthesis of the H\(^+\)-ATPase subunits c, b and δ are determined at the level of translational initiation. This conclusion is based primarily upon the observed effects of changes in the DNA sequences upstream of the cloned ap genes upon their rates of translation. It should also be borne in mind that the large differences in the levels of expression in vitro between the c, b and δ subunit genes were observed even at the very lowest DNA concentrations. This therefore also means that the differences in synthesis rates were maintained under conditions where there cannot have been limitation of the elongation rates as the result of scarcity of factors required for polypeptide synthesis.

Most striking is the highly efficient translation of the subunit c cistron, which is dependent upon the presence of a long intercistronic sequence upstream of its start codon. The removal of the sequence >20 bp upstream of the c subunit Shine-Dalgarno sequence reduced the synthesis of this subunit by a factor of ~5 (lane 5, Figure 3 and Table I; compare also lane 3, Figure 4), and further shortening of this sequence led to much larger reductions in synthesis. The truncation of the intercistronic sequence lying before the b subunit gene (where the intercistronic sequence upstream of the c subunit had already been removed) up to 9 bp before the Shine-Dalgarno sequence by contrast, had no effect on the expression of this gene (compare lanes 10 and 11, Figure 3). The rate of translation of the subunit b gene was however affected by the presence of the intercistronic sequence lying upstream of the c subunit gene (see below). Since similar reductions in expression of the subunit c gene upon the shortening of its upstream intercistronic sequence were also observed using an entirely different expression plasmid (pLfl101), the observed effects cannot be associated with any specific local structure of either plasmid lying near the cloning site.

The codons of the genes encoding subunits c, α and β of the E. coli H\(^+\)-ATPase (unlike the codons of the genes encoding subunits γ, δ and ε) conform to the types commonly found in highly expressed E. coli genes (Futai and Kanazawa, 1983; Grantham et al., 1981). These "optimal" codons generally correspond to the most abundant iso-accepting tRNA species (Ikemura, 1981a, 1981b), and they may allow codon/anticodon interactions of intermediate energies which are postulated to be conducive to rapid turnover (Grantham et al., 1981; Grosjean and Fiers, 1982). However, the data presented here contradict the proposal that codon usage directly determines the relative rates of translation of ap genes, and rather suggest that the above observations bear a different significance.

In Figure 6 the sequences of the translational initiation regions of the c, b and δ subunit genes are presented, together with the average (postulated as "ideal") sequence for a translational initiation site deduced from analysis of the sequences of different genes (Scherer et al., 1980; Gold et al., 1981). The sequences of the three ap genes are generally dissimilar.
The translational initiation region of the subunit c gene and the intercistronic sequence upstream of the subunit c gene were intercistronic sequence is responsible for promoting efficient ribosomal initiation. Sequences showing striking similarity to than purely the physical size or secondary structure of the stability of local mRNA secondary structures such as the ones mentioned above. It is not clear why similar experiments per­
formed with the plasmids described here yielded much smaller, and interpreted this as the result of changes in the stoichiometry of synthesis of subunits of the lower, and found in the translational initiation regions of other efficiently translated genes in E. coli (and in particular of ribosomal protein genes) and bacteriophage λ. Four of these regions are compared in Figure 7.

The possibility that the identified sequence pattern acts as an extra signal that enhances the rate of translational initiation, playing a particularly important role in polycistronic mRNAs, is attractive but needs to be thoroughly tested by experiment. A strong influence upon translational initiation of sequences many bases upstream from the Shine-Dalgarno sequence has also been observed in other systems (see, e.g., Kastelein et al., 1983; Roberts et al., 1979) where a sequence of the subunit c gene type was not present. However, direct evidence that the subunit c type sequence can act more generally to enhance translational initiation has come from the cloning of the subunit c intercistronic sequence in series with the human interleukin 2 and β-interferon genes (McCarthy et al., in preparation). The expression of the subunit b gene in the pBl050(cb) type plasmids is apparently linked to the expression of the preceding subunit c gene. This phenomenon is reminiscent of the 'transla­
tional coupling' effect identified for example in studies of the E. coli ribosomal protein operons (Nomura et al., 1984). The parallel rise in expression of the subunit b and c genes of pBl050(cb) with increasing DNA concentration indicates that ribosomes re-initiate at the subunit b cistron. The low frequency of re-initiation could be determined partially or
completely by the two adjacent stop codons of the subunit c cistron, the 58-8 bp intercistronic sequence upstream of the subunit b cistron, or the possible secondary structure in this region. The poor expression of the subunit δ gene obtained with pSE1(612) may also reflect the loss of some translational coupling with the subunit b cistron.

It is important to consider how well the relative efficiencies of translational initiation of the b, e and δ subunits correspond to the amounts of each of these subunits that are required for assembly of the E. coli H^+-ATPase. The synthesis of subunits b and c can be directly compared using the pB1050(cb) type plasmids, and showed a molar stoichiometry of synthesis in the range 6 ~ 10, which compares favourably with the estimated stoichiometry of the H^+-ATPase (Foster and Fillingame, 1982; von Meyenburg et al., 1982b). The molar stoichiometry of synthesis of subunits c and δ, on the other hand, can only be estimated more indirectly. Comparison of the expression of pSE1(612), pHB6(b1) and the subunit c gene and pB1050(cb) type plasmids indicates that the ratio of translational initiation efficiency for subunit c:subunit δ is at least 18. By analogy to the effect of re-initiation upon expression of the subunit b gene, it might be argued that this ratio is exaggerated because the δ subunit gene in pSE1(612) is no longer located downstream of the b subunit gene.

Finally, the major factor underlying the synthesis of the appropriate amounts of the b, c and δ subunits of the E. coli H^+-ATPase has been shown to be the efficiency of translational initiation. The subunit stoichiometry of the other subunits may be achieved in the same way.

Materials and methods

Bacterial strains

MCG1 was constructed by transferring a mutated recA sequence from JC10240 (Hfr P045 sol 300; Tn10 recA56 thr 318 pyrE300 recA1 thi-1) to the E. coli K12 lac repressor overproducer strain JM103 (Δ(lac pro) thi ara supE endA sbcC hsdR') F' traD86 proAB lacY ΔZaM15, Messing et al., 1981) using the general transducing bacteriophage P1. In order to construct recA/exon ("exc") derivatives of JM103, an exc' derivative of this strain was first of all isolated after mutagenesis with N-methyl-N-nitro-N-nitrosoguanidine and penicillin enrichment. iv was used as a marker for the transfer of defective recA sequences from strains DG27/10 and DG27/9 (Ap^E') and DG27/9 (Ap^E) (Schauer et al., 1976) via P1 transduction. The exc' derivatives isolated in this way were rendered recA ("as described") thus producing strains MCG2 (Ap^E') and MCG3 (Ap^E). The MCG strains 1 - 3 were used for transformation (Dagert and Ehrlich, 1979; Banahan, 1983) and complementation tests involving derivatives of pDR540. Transformations with pL101 (or derivatives thereof) were performed using strains N996A and N4830 (Pharmacia P-L. Biochemicals).

Expression of genes from the E. coli ap operon

Cells of strain MCG1 containing the desired plasmids were allowed to grow in minimal medium at 37°C for 20 min in a shaker. The cells were harvested by centrifugation, and washed twice with ethanol and diethylether. The dependence of radioactive incorporation into protein upon time was linear during the stated incubation times. Labelled proteins synthesized in the in vitro system were separated on 15.5% SDS-polycrylamide gels (with 3% stacking gels), which were subsequently stained with Coomassie Brilliant Blue R, impregnated with 2,4-diphenyloxazol (Banner and Laskey, 1974) and dried. The positions of the radioactively labelled proteins on the gels were checked against the positions of the components of proteins of standard F. coli H^+-ATPase. Autoradiography was performed with Kodak X-Omat AR or Agfa-Gevaert Curix X-ray film. Superimposition of the exposed and developed films on the dried gels allowed the precise excision of particular bands in equally sized segments for radioactivity analysis in scintillant. Control experiments in which serial dilutions of cultures were loaded onto gels showed that the radioactivity measured in the gel sections was linearly related to the amount of radioactive protein in the bands. Corrections of the measured radioactive incorporation into a specific protein band for background in the gels were made by subtracting the radioactivity activity measured in equally sized sections cut from corresponding positions in control lanes which lacked this particular protein band. For example, for correction of the measured radioactive activity in a gel section containing the subunit b band, the synthesis of which was directed by pB1050(cb), an equally sized section was excised from the equivalent position in a lane loaded with the synthesis products of pH116(c); pDR540 was correspondingly used as the control plasmid for pHB6001(c), pB1050(cb) and pSE1(612), respectively. Identical concentrations of the experimental and control plasmids were used in the titrations.

In vivo pulse-labelling

Cells of strain MCG1 containing the desired plasmids were allowed to grow in minimal medium at 37°C to a density of 0.5, at which point isopropyl-β-D-thiogalactoside (final concentration 2 mM) was added. After a further 20 min, 15 μg of [35S]-methionine (specific radioactivity 1000 Ci/mmol) was added to 1 ml of these cells and incubation was continued for another minute. Then L-methionine (final concentration 1 mM) was added and after a further minute's incubation the cells were rapidly cooled to 20°C. After thawing, centrifuging, and washing twice with 70 mM Tris-HCl (pH 7.5), 10 mM

525
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