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Phosphotransferase-mediated regulation of carbohydrate utilisation in *Escherichia coli* K12: identification of the products of genes on the specialised transducing phages λ*iex (crr)* and λ*gsr (tgs)*

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The expression of genes adjacent to *ptsI* was investigated using a series of specialised transducing phages carrying different, overlapping, segments of the *cysA-gsr-ptsI-ptsH-iex-cysZ-lig* region of the genome of *Escherichia coli*. The polypeptides were synthesised following the infection of u.v.-irradiated lysogenic and non-lysogenic *uvrA recA* hosts or a *uvrA recA* host carrying the λ*cI*$^+$ plasmid pKB280. The polypeptides were identified by SDS-polyacrylamide gel electrophoresis and fluorography. The *gsr* gene product had a mol. wt. of 23 000. The product of the *iex* gene was tentatively identified as a protein of mol. wt. of either 33 000 or 21 000. Hpr, the product of the gene *ptsH*, had a mol. wt. of 9000. The *gsr* gene appeared to be expressed at a higher level in a non-immune host, which suggests that it was transcribed from λ promoters. A new λ host strain, suitable for the detection of small polypeptides (mol. wt. <30 000) is described.
Key words: Escherichia coli/phosphotransferase/inducer exclusion/lambda/*iex* and *gsr* gene products

Introduction

The phosphoenolpyruvate-dependent phosphotransferase system (PTS) effects the uptake and phosphorylation of a number of sugars by *Escherichia coli* (Kundig *et al.*, 1964). These sugars, generically termed 'PT-sugars' (Roseman, 1969), include glucose, fructose, hexitols, and *N*-acetylglucosamine. On the other hand, pentoses and disaccharides are taken up by active transport and are phosphorylated by ATP-linked kinases only after they have entered the cell: these are generically termed 'non PT-sugars'.

The PTS consists of an enzyme I, specified by the gene *ptsI*, that catalyses the transfer of phosphate from phosphoenolpyruvate to (usually) a small histidine-containing protein HPr (specified by the gene *ptsH*). Membrane-associated and sugar-specific enzymes II catalyse the uptake and concomitant phosphorylation (with phospho-HPr as phosphate donor) of the appropriate sugars; for this phosphorylation to occur, additional carriers ('factors III') may be required to act between phospho-HPr and the transport protein in the membrane (Roseman, 1972). Such a factor III specific for the uptake of methyl α-glucoside and of (the majority of) glucose, specified by a gene designated *crr* in *Salmonella typhimurium* (Saier and Roseman, 1972) has also been recognized in *E. coli* although it was originally designated *tgs* in that organism (Kornberg and Watts, 1978).

An additional gene, whose product plays an important role in regulating the response of *E. coli* to mixtures of PT- and non PT-sugars, was recognized by Jones-Mortimer and Kornberg (1974). It was long established that mutants of *E. coli* that lacked either enzyme I activity (Gershanovitch *et al.*, 1967; Wang *et al.*, 1970) or HPr (Gershanovitch *et al.*, 1977) grew on non PT-sugars only after a prolonged lag. Jones-Mortimer and Kornberg (1974) isolated from one such mutant, which carried a temperature-sensitive gene specifying enzyme I function (and that was therefore PtsI$^+$ at 30°C but PtsI$^-$ at 40°C), further mutants that were able to adapt rapidly to growth on a variety of non PT-sugars at 40°C: by analogy with apparently similar *crr*-mutants of *S. typhimurium* (Saier and Roseman, 1972), these *E. coli* mutants were also designated *crr*. However, it has now become clear that these latter mutants differ in many respects from *crr*-mutants of Salmonella (Kornberg *et al.*, 1980; Parra *et al.*, 1982) and that the gene notation employed is both misleading and confusing. Parra *et al.* (1982) therefore suggested that the gene that specifies the glucose-specific factor III and that also affects the activity of adenylate cyclase be termed *gsr* (glucose-specific repression), whereas the gene that plays a role in the exclusion by PT-sugars of inducers of active transport systems be termed *iex* (inducer exclusion). We adopt this nomenclature in this paper.

We reported previously the isolation of specialised transducing phages carrying segments of the *E. coli* genome from *cysA* to *lig*, and construction of a map of restriction sites in this DNA (Britton *et al.*, 1982). Here we report the analysis of the proteins specified by the specialised transducing phages, using u.v.-irradiated cells, and identification of the proteins specified by the *E. coli* genes on these phages.

To achieve the identification of the small polypeptides, we have developed a new λ host strain, PB12 (pKB280). This strain was more suitable for these post-irradiation infection experiments than was the standard host, 159 (λ*ind*$^-$), or PB11 (λ*ind*$^-$) for two reasons: its higher content of λ immunity repressor completely abolished the synthesis of most λ proteins, and its enhanced u.v. irradiation sensitivity eliminated the production of small, host-specified, polypeptides.

Results and Discussion

Identification of polypeptides specified by genes carried on λ

To identify the polypeptides that are specified by the genes carried on the specialised transducing phages isolated by Britton *et al.* (1982), u.v.-irradiated cells of strains PB11, PB11 (λ*ind*$^-$) and PB12 (pKB280) were infected with the phage. [Since PB11 (λind$^-$) and PB12 (pKB280) contain λ immunity repressor, λ protein synthesis is depressed relative to that from *E. coli* promoters.] The radioactive polypeptides synthesised after infection were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by fluorography. The results of these experiments are shown in Figures 1-4 and summarised in Table I.

The ptsH gene product, Hpr. The specialised transducing phages λJM29, λJM109, λJM121, and λJM133 expressed a polypeptide of mol. wt. 9000 (Figures 1-4) that was not expressed by the wild-type phage λ*cI857 S7*. Each of these

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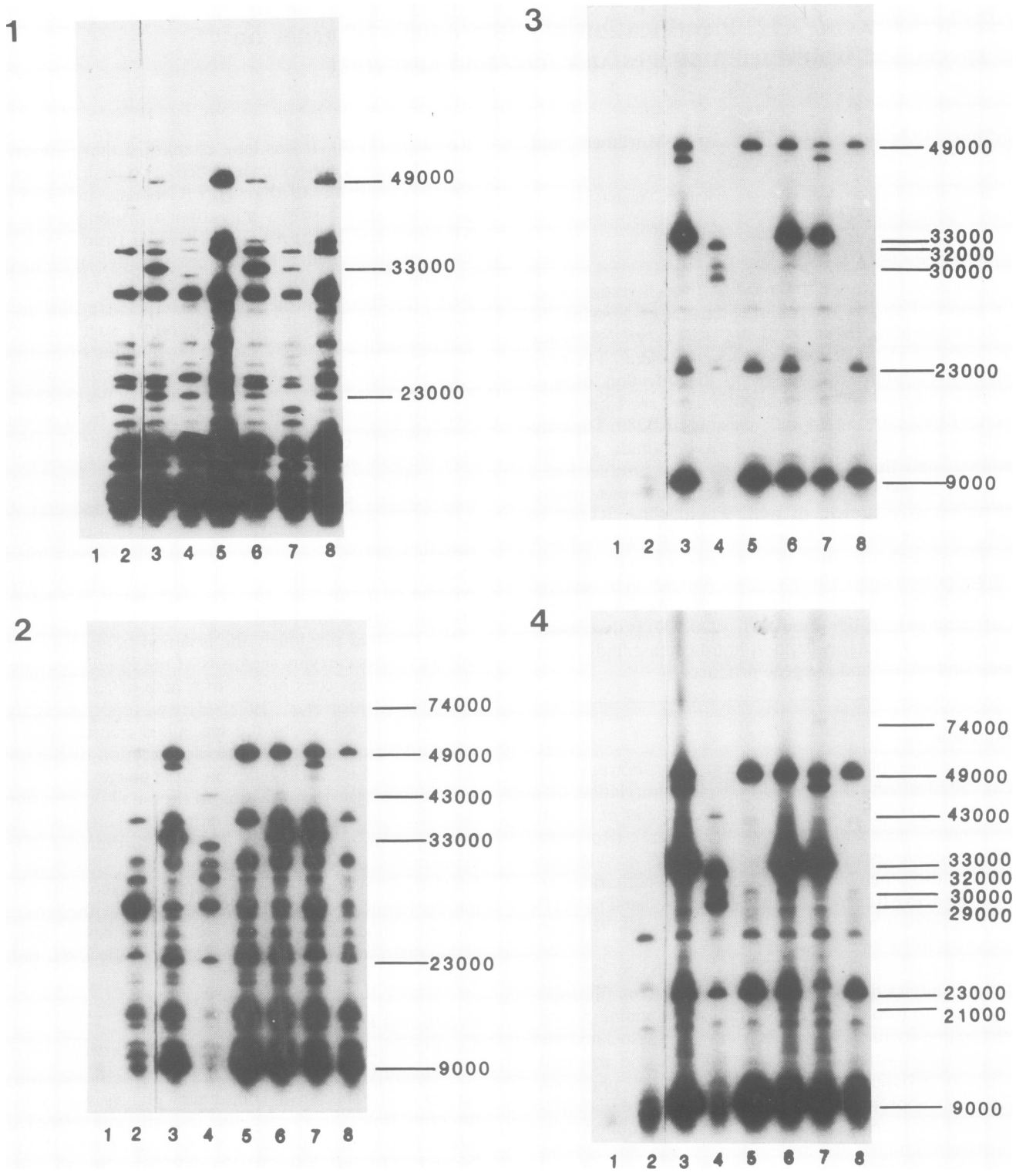

Fig. 1–4. Fluorograms of the [^{35}S]methionine-labelled polypeptides formed after infection of u.v.-irradiated λ-sensitive host strains: **Fig. 1.** PB11, **Fig. 2.** PB11 (λ*ind*$^-$), **Fig. 3.** PB12 (pKB280) 18 h exposure, and **Fig. 4.** PB12 (pKB280) 5 days exposure. In each Figure **track 1** shows pattern of the uninfected host strain; **track 2** λ*cI857 S7*; **track 3** λ*JM29;* **track 4** λJM106; **track 5** λJM109; **track 6** λJM121; **track 7** λJM133; **track 8** λJM109 obtained from the lysogen FP23.

phages has been shown (Britton *et al.*, 1982) to be capable of transducing the *ptsH*$^+$ wild-type allele: the Hpr protein specified by this gene is known to have a mol. wt. of 9022, determined from its amino acid composition (Weigel, 1978). The phage λJM106, which does not complement *ptsH* lesions (Britton *et al.*, 1982), did not express the 9000 mol. wt. polypeptide.

The gsr gene product, factor III^{Glc}. The specialised transducing phages λJM106, λJM29, λJM109, and λJM121 were found to express a polypeptide of mol. wt. 23 000, (Figures

Table I. Identification of the polypeptides encoded on the specialised transducing phages

Phage	Lesions corrected										
	cysA		*gsr*		*ptsH*		*iex*		*cysZ*		*lig*
λJM106	+		+								
λJM109			+		+		(+)				
λJM121	+		+		+		+		+		
λJM29			+		+		+		+		
λJM133					+		+		+		+
	Polypeptides expressed (mol. wt. x 10^{-3})										
	41	29	32	30	23	49	9	33	21	47	74
λJM106	+	+	+	+	+						
λJM109					+	+	+				
λJM121				+	+	+	+	+	+		
λJM29					+	+	+	+	+	+	
λJM133						+	+	+	+	+	+

(+) indicates that the phage can correct the lesion, but either did not complement it or failed to express a polypeptide of the required mol. wt.

1–4), which was not expressed by the wild-type phage. Each of these phages is capable of transducing the *gsr*$^+$ allele (Britton *et al.*, 1982). The phage λJM133 did not complement *gsr* mutants (Britton *et al.*, 1982) nor did it express the 23 000 polypeptide. In view of the demonstrations (Scholte *et al.*, 1981) that factor III^{Glc} of *S. typhimurium* has a mol. wt. of 21 000, and that the *gsr*-gene specifies factor III in *E. coli*, the identity of the product with factor III^{Glc} cannot be seriously doubted.

The cysA gene product. The specialised transducing phages λJM106 and λJM121 expressed a polypeptide of mol. wt. 30 000 that was not expressed by the other phages. Since both these phages transduce the *cysA* gene, this polypeptide is likely to be the product of this gene. If however an *E. coli* gene corresponding to the *S. typhimurium cysM* gene lies between *ptsI* and *cysA*, the polypeptide could be its product. If so, λJM121 cannot carry the complete *cysA* gene.

The iex and cysZ gene products. The specialised transducing phages λJM29 and λJM133, known to transduce the *iex*$^+$ wild-type allele into *iex* mutants (Britton *et al.*, 1982), expressed polypeptides of mol. wts. 33 000, 21 000, and 47 000 (Figures 1–4) which were not expressed by the wild-type phage. These polypeptides were not expressed by λJM106, which does not correct *iex* lesions.

The phage λJM121 expressed the polypeptides of mol. wts. 33 000 and 21 000 but not that of 47 000. All three phages transduced appropriate strains to Cys$^+$ and Iex$^+$, but it is not certain that, in all cases, the transductants were still diploid. If they were, the products of the *iex* and *cysZ* genes are the 33 000 and 21 000 mol. wt. polypeptides; if not, one of the functions might have been ascribed to the 47 000 mol. wt. polypeptide. However, further experiments enabled us to show that this larger polypeptide was not the product of the *iex*$^+$ gene.

λJM109 expressed none of these three polypeptides. A lysogen of λJM109 (strain FP23) in a *ptsH iex* host (strain FP14) showed the Iex$^+$ phenotype (Britton *et al.*, 1982). Phage of the correct genotype and restriction pattern could be recovered from this lysogen, by thermal induction, but the original host was not recovered by heat-pulse curing. The phage recovered from the lysogen did not express the 33 000 or 21 000 mol. wt. polypeptide. The prophage was integrated in the *ptsI* region of the chromosome since an induced lysate transduced *cysZ* (a linked gene not present in λJM109) at a frequency of one transductant per 1.3 x 10^9 plaque-forming unit (p.f.u.) but did not transduce *galK*, a gene linked to *att*λ. Presumably the lysogen contained an *iex*$^+$ gene that resulted from an intragenic recombination event on lysogenisation. To avoid the effects of intragenic recombination, strain PB15 (*iex recA*) was infected with λPB4 (an *S*$^+$ revertant of λJM109) and the helper phage λ*imm21*, together with λ*imm21 b2* and λ*ch80 del9* to select for lysogens of λPB4. The lysogenic derivatives of PB15 were shown to be true lysogens by thermal induction of the phage at 42°C, and were still Iex$^-$. The phage λPB4 was shown to transduce a *ptsH* mutant to PtsH$^+$.

From these results it appears that λPB4 is unable to transduce the *iex*$^+$ wild-type allele into PB15. Since λJM109 was able to correct the *iex* lesion of strain FP14, it appears that λJM109 or λPB4 carry part, but not all, of the *iex* gene, and therefore that the *iex* gene must lie at the left-hand end of the three genes specifying the polypeptides of mol. wts. 33 000, 21 000, and 47 000. Furthermore, the gene for the 47 000 mol. wt. polypeptide must lie at the right-hand end of this group of three genes, since it is not expressed by λJM121. Therefore the 47 000 mol. wt. polypeptide cannot be the product of the *iex* gene.

A polypeptide of mol. wt. 49 000. The specialised transducing phages λJM29, λJM109, λJM121, and λJM133 were shown to express a polypeptide of mol. wt. 49 000 (see Figures 1–4) which was not expressed by the wild-type phage. From the method of construction of these phage (Britton *et al.*, 1982) it is known that some part of the gene *ptsI* must be present in the cloned DNA. Kukumyinka *et al.* (1982) have shown that the Enzyme I from *S. typhimurium* has a mol. wt. of 57 000. The specialised transducing phage λJM106, shown by restriction analysis (Britton *et al.*, 1982) not to contain the same part of the *ptsI* gene as is present in the above phages, did not express the polypeptide of mol. wt. 49 000: it is likely that this polypeptide represents a fragment of Enzyme I.

Other proteins expressed by the λ phages. As can be seen from Figures 3 and 4 [the expression of the phages in PB12 (pKB280)] there are several other polypeptides expressed. It is unlikely that the polypeptides are derived from λDNA since they are absent from several phages. These polypeptides of mol. wts. 29 000, 32 000, and 41 000 are expressed by λJM106 only, but their identity is as yet unknown. It is possible that they are products of other genes involved in sulphate transport: the *cysA* locus of *S. typhimurium* consists of three cistrons (Mizobuchi *et al.*, 1962).

The specialised transducing phage λJM133 was the only one to specify a polypeptide of mol. wt. 70 000–80 000 and to correct a lesion in the DNA ligase gene, *lig*. Since the mol. wt. of *E. coli* DNA ligase is known to be 74 000 (Modrich *et al.*, 1973) it is probable that this polypeptide is the lig$^+$ gene product.

Conclusions

The results presented serve to identify the polypeptides specified by the genes *cysA*, *gsr*, and *ptsH*, which are of mol. wts. 30 000, 23 000, and 9000, respectively. They leave unresolved the question whether the product of the *iex* gene is a polypeptide of mol. wt. 33 000 or 21 000. The *cysK* gene of *E. coli* and *S. typhimurium* also maps in this region of the genome (Fimmel and Loughlin, 1977; Wiater and Hulanicka, 1979; Hulanicka and Klopotowski, 1972; Hulanicka *et al.*,

Table II. Organisms and plasmid used

E. coli strains	Genotype	Origin
CSH25	*supF thi pro*	Miller (1972)
159	*uvrA*	J.Scaife
PB11	*uvrA recA srl*::Tn10	P1.*recA srl*::Tn10→159
PB11 (λ*ind*$^-$)	*uvrA recA srl*::Tn10 (λ*ind*$^-$)	lysogenisation of PB11 with λ*ind*$^-$
PB12 (pKB280)	*uvrA recA* (pKB280)	P1 transduction of PB11 to *srl*$^+$ followed by transformation with pKB280
FP14	*ptsI*^{ts} *ptsH iex umgC metB*	Britton *et al.* (1982)
FP23	FP14 (λJM109)	This paper
PB15	*thyA ptsI iex cysZ* Str^R *recA srl*::Tn10 F$^-$	P1.Str^R and P1.*recA* sr1::TN10→JM1766
JM1766	*thyA ptsI iex cysZ* F$^-$	Britton *et al.* (1982)
Plasmid		
pKB280	Tc^R λ*cI*$^+$ (λ*cI* is expressed from the *lac* promoter in this plasmid)	Bachman and Ptashne (1978)
λ		
λwt	*cI857 S7*	P.Oliver
λJM29	*cI857 S7 xis6 nin6 b515 b519 gsr*$^+$ *ptsH*$^+$ *iex*$^+$ *cysZ*$^+$	
λJM106	*cI857 S7 xis6 nin5 b515 b519 cysA*$^+$ *gsr*$^+$	
λJM109	*cI857 S7 xis6 nin5 b515 b519 gsr*$^+$ *ptsH*$^+$	Britton *et al.* (1982)
λJM121	*cI857 S7 xis6 nin5 b515 b519 cysA*$^+$ *gsr*$^+$ *ptsH*$^+$ *iex*$^+$ *cysZ*$^+$	
λJM133	*cI857 S7 xis6 nin5 b515 b519 ptsH*$^+$ *iex*$^+$ *cysZ*$^+$ *lig*$^+$	
λPB4	*cI857 xis6 nin5 b515 b519 gsr*$^+$ *iex*$^+$	*S*$^+$ derivative of λJM109
λ*imm21*		P.Oliver
λ*imm21 b2*		B.Wilkins
λ*ch80 del9*		P.Oliver
λ*ind*$^-$		P.Oliver

1974). Its product, O-acetylserine sulfhydrase A, has a mol. wt. of 34 000 (Becker *et al.*, 1969). A polypeptide of mol. wt. 33 000 expressed by our phages could well be the product of the *cysK* gene. Either the polypeptide of mol. wt. 21 000 or another of 47 000, is the product of the *cysZ* gene. However, Buxton *et al.* (1980) showed that the *xap* gene specifying xanthosine phosphorylase lies ~5 kb from the *ptsI* gene (to the right-hand side in our maps). The gene product of *xap* has a mol. wt. of 205 000 (±10 000) (Hammer-Jesperson *et al.*, 1980). If the native enzyme consists of four identical subunits, it is possible that the polypeptide of mol. wt. 47 000 is the subunit of xanthosine phosphorylase. Thus it appears that there may be more genes in this region of the chromosome than there are polypeptides detectable by labelling with [^{35}S]methionine. If so, either one of the polypeptides may contain no methionine, or one of the bands observed in Figure 1 may constitute a doublet. These possibilities are being investigated.

Identification of some of the polypeptides described in this paper was made possible only by using the λ host strain PB12 (pKB280), which carried the λ immunity repressor on the plasmid. Since this plasmid is known to specify the synthesis within the host strain of the repressor to such an extent that it may form 1% of all protein present (Reeve, 1978), use of this strain will greatly reduce the expression of λ proteins and will thus facilitate detection of cloned *E. coli* DNA. This presents considerable advantages over the use of the commonly-employed lysogenic λ host strains, either 159 (λind$^-$) or the more u.v.-sensitive derivative PB11 (λ*ind*$^-$); in these strains, the polypeptides specified by cloned genes may be obscured on the gels by the various λ proteins that are also expressed. Strain PB12 (pKB280) is especially suitable for the identification of polypeptides of mol. wt. <30 000.

Materials and methods

L-[^{35}S]methionine was obtained from Amersham International plc, UK. Acrylamide and N',N-bis-methyleneacrylamide were of a grade especially purified for electrophoresis; similarly, SDS was of a grade specially purified for biochemical work. These chemicals, together with polyacrylamide (mol. wt. >5 x 10^6) were obtained from BDH Chemicals Ltd., UK. All other chemicals were of analytical grade.

The *E. coli* and phage strains used are listed in Table II, as are the plasmid genotypes.

Preparation and purification of λ phage

The host strain (CSH25) was grown to a density of 6 x 10^8 cells/ml at 37°C in 500 ml of LB containing 10 mM $MgCl_2$. The appropriate λ phage was added at a multiplicity of infection (m.o.i.) of 0.5 and lysis of the host cell was monitored turbidimetrically. Any unlysed cells were burst by addition of 1 ml of $CHCl_3$ and shaking for 15 min at 37°C. The cell debris was removed by centrifugation (23 400 *g* for 10 min in the SS-34 rotor of a Sorvall RC5B centrifuge). The phage suspension was made 1.0 M with NaCl and the DNA and RNA were digested by the addition of 1.0 mg of DNase and 1.0 mg of RNase and incubating at room temperature for 1 h. The phage were precipitated, with 10% (w/v) polyethyleneglycol 6000 according to the method of Yamamoto *et al.* (1970). The precipitated phage were centrifuged (23 400 *g*, 10 min, Sorvall RC5B centrifuge) and were then resuspended in 50 ml of 10 mM Tris-HCl and 10 mM $MgCl_2$, pH 7.4. After a further centrifugation at 47 800 *g* for 3 h in the Sorvall RC5B centrifuge, the phage were purified by the CsCl block gradient method described by Davies *et al.* (1980).

Post-infection labelling

Cultures of PB11, PB11 (λ*ind*$^-$), or PB12 (pKB280) were grown to a density of 10^9 cells/ml in K-medium (Rupp *et al.*, 1971) containing 10 mM maltose in all cases plus 10 μg of tetracycline (Tc)/ml for PB12 (pKB280). The cells were washed twice and resuspended to 2 x 10^9 cells/ml in sulphur-free Hershey salts (Worcel and Burgi, 1974) containing 10 mM maltose. Samples (5 ml) were irradiated with u.v. light (450 J/m) for 3 min and stored in the dark at 0°C for 1 h to prevent photo-reactivation of the host DNA. Samples (200 μl) of irradiated cells were made 10 mM $MgCl_2$, 2 mM cAMP, and 50 μg of D-cycloserine/ml and were infected with purified λ phage at a m.o.i. of 8 (4 μl of 10^{11} p.f.u./ml). The samples were incubated at 37°C for 20 min for phage adsorption. Labelling of the phage encoded proteins was carried out at 37°C with L-[^{35}S]methionine [final concentration 10 μCi/ml (0.37 MBq/ml)] either for 100 min using PB11, or 180 min using PB11 (λ*ind*$^-$) or PB12 (pKB280). After labelling, L-methionine was added to a final concentration of 800 μg/ml and the cells were incubated at 37°C for 10 min. The cells were centrifuged and solubilised in 20 μl SDS-dissociating buffer (see below) and analysed by SDS-PAGE using 12–20% gradient gels (see below). The polypeptide bands were identified by fluorography (see below).

SDS-PAGE

The polyacrylamide gel electrophoresis system used (Britton, 1981) was a modified version of the discontinuous buffer system described by Laemmli (1970). The gel was cast and run in a system similar to the one described by Studier (1973). The separating gel consisted of an acrylamide gradient (12–20%), produced from a stock acrylamide solution containing acrylamide and N,N'-bis-methyleneacrylamide in the ratio 30.0%:0.8% (w/v). The gel also contained, in addition to the components described by Laemmli (1970), 0.16% polyacrylamide and 2 mM disodium EDTA. The polyacrylamide (uncross-linked) was used to strengthen the gels and help prevent cracking during the drying-down process, as described by Douglas and Butow (1976). The unpolymerised separating gel was overlayed with water-saturated butan-2-ol to give a straight edge as described by Neville (1971). The stacking gel contained 3.7% acrylamide (using the same stock as for the separating gel). The stacking gel and electrode buffers were as described by Laemmli (1970), except for the addition of 2 mM disodium EDTA. The dissociation buffer contained 2 mM disodium EDTA, 2.5% (w/v) Ficoll type 400, and 2.5% (w/v) 2-mercaptoethanol in addition to the constituents described by Laemmli (1970). The samples were fully solubilised by boiling for 3 min. All gels were routinely run overnight, at room temperature, at a constant voltage of 80 V (5.3 V/cm). After electrophoresis, the gels were fixed in a solution containing

4% (w/v) 5-sulphosalicylic acid and 10% (v/v) acetic acid, for 15 min at 55°C; they were stained in 0.5% (w/v) Coomassie Brilliant Blue-R, 45% (v/v) methanol and 10% (v/v) acetic acid at 55°C for 15 min. Gels were destained in 5% (v/v) methanol and 8% (v/v) acetic acid at 55°C using polyurethane packing foam to adsorb excess dye. In order to detect the radioactively labelled proteins by fluorography the gels were thoroughly washed in distilled water to remove any acid and were impregnated with 0.8 M sodium salicylate, as described by Chamberlain (1979). The gels were dried down, using a Bio-Rad 2214 gel drier, onto Whatman 3 MM paper; they were then exposed to preflashed Fuji RX X-ray film at −80°C for 18 h as described by Laskey and Mills (1975). The following marker proteins were used: bovine serum albumin (68 000), bovine γ-globulin (50 000 and 23 500), ovalbumin (43 000), myoglobin (18 500), and lysozyme (14 300).

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