IDENTIFICATION AND CHARACTERISATION OF A TRANSDUCING PHAGE CONTAINING THE GENE FOR RIBOSOMAL PROTEIN S1 OF ESCHERICHIA COLI

JONATHAN CHARLES STIRLING NOBLE

CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

IDENTIFICATION AND CHARACTERISATION OF A TRANSDUCING PHAGE CONTAINING THE GENE FOR RIBOSOMAL PROTEIN S1 OF *ESCHERICHIA COLI*

by

© Jonathan Charles Stirling Noble, B.Sc.

A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Division of Basic Medical Sciences
Faculty of Medicine
Memorial University of Newfoundland

June 1982

St. John's Newfoundland

Abstract

The recent mapping of the gene for ribosomal protein S1 (*rpsA*) at 20 minutes on the *E. coli* chromosome (Ono, M., Michihiko, K. & Mizushima, S. (1979) Mol. Gen. Genet. 174, 11-15), has been exploited in studies aimed at the identification of a transducing phage carrying this locus. The coding capacities of a λ*aroA* transducing phage containing a portion of this chromosomal region (R. Weisberg, personal communication), have been analysed in an UVL irradiation and infection system by a combination of techniques of immunoprecipitation and SDS and two dimensional polyacrylamide gel electrophoresis. This analysis has indicated that among the six or so bacterial proteins encoded by this phage, one comigrating with pure S1 at pI 5-6 and M_r = 70,000 reacts specifically with antiserum developed against the purified protein. This species which is identified as ribosomal protein S1 by these criteria, appears to be expressed under the direction of a bacterial promoter.

A detailed restriction map of the genome of the λ*aroA* transducing phage has been constructed from the results of numerous single and double enzyme digestions. This information has permitted an analysis of the relationship between the arrangement of bacterial DNA sequences in the inse-

rt contained by the transducing phage and that of the chromosomal region from which it was derived. The similarity of structure indicated by this analysis supports identification of the bacterial activity governing expression of *rpsA* in the phage context as the normal chromosomal promoter for this gene.

Acknowledgements

I would like to thank first of all my supervisor, Professor Bruce Sells, who provided both invaluable advice and much needed encouragement during the course of this work. In addition I wish to thank Dr Stephen Boyle and Dr Banfield Younghusband, and among my graduate student colleagues, Anthony Metcalfe, Fred Jacobs and Jonathan Wright, for their contribution to this work specifically and more generally to the helpful environment I enjoyed as a student in the Division of Basic Medical Sciences.

Lastly, I would like to thank Dr Kazuo Adachi and Professor Robert Weisberg (NIH) for their generous gifts of anti S1 serum and λ*aroA* transducing phages respectively.

Table of Contents

Table of Contents (cont'd)

Table of Contents (cont'd)

List of Tables

List of Figures

List of Figures (cont'd)

List of Abbreviations

UVL	:	ultraviolet light
r-protein	:	ribosomal protein
rRNA	:	ribosomal RNA
mRNA	:	messenger RNA
SDS	:	sodium dodecyl sulphate
$(Na)_2$ EDTA	:	ethylene diamine tetraacetic acid (disodium salt)
NP-40	:	Non-Idet P40
BSA	:	bovine serum albumin
TEMED	:	N,N,N',N'-tetramethyl ethylenediamine
tris	:	tris(hydroxymethyl) aminoethane
kbp	:	kilobase pairs

INTRODUCTION

Background

The bacterial ribosome possesses a complex structure, containing in E. coli a total of fifty-three unique r-proteins and three different rRNAs. The accumulation of these ribosomal components exhibits both a strict coordination among individual species (1), and a collective sensitivity to demands of cellular growth rate (2) and conditions of amino acid starvation (3).

Our understanding of the mechanisms underlying this regulated expression of ribosomal genes, both r-proteins and rRNA, has improved considerably in recent years (see 4) largely as a result of the development and use of a variety of novel genetic methods. These methods have depended to a large degree upon the availability of ribosomal genes isolated in the form of specialised λ transducing phages. The main advantage afforded by the use of these phages has been their freedom from the problems posed to genetic analyses by the ribosome's complexity and indispensability for cell viability.

The approaches taken in the isolation of these transducing phages and the range of contexts in which they have proved valuable is discussed below in reference to the

study of r-protein genes.

Transducing phages containing ribosomal protein genes

Isolation of transducing phages

The creation of λ transducing phages containing a particular bacterial locus occurs in most instances only as a rare event, owing to a requirement for two in themselves rare occurrences; phage integration at a chromosomal site close to the gene of interest, and aberrant excision of the prophage from this site to produce a phage including an appropriate segment of bacterial DNA. The frequency of aberrant excision of λ prophage is generally of the order of 10^{-4} per induced lysogenic cell (5). The frequency of prophage integration at sites around the chromosome varies greatly from site to site but is generally less than 10^{-3} of the frequency of integration at the primary λ integration site (attλ) (5,6). The combined rareness of these two events has usually demanded that powerful selection methods be available to allow isolation of transducing phages containing particular loci (5).

The lack of selectable mutant phenotypes has in most instances precluded the use of direct selection methods in the isolation of phages containing r-protein genes. Instead, indirect approaches to selection have had to be adopt-

ed based upon the knowledge where available of the position of the desired locus in relation to surrounding selectable chromosomal markers. The selection methods employed in these indirect approaches have involved two overall strategies. In the first, lysogens containing prophage integrated at points proximal to the locus of interest have been selected (7,8). In the second, phages containing bacterial DNA from the desired chromosomal region have been selected from a mixed lysate (7,9-11). Both strategies have invariably included use of host strains deleted for attλ, in which the relative frequency of rare lysogens is enhanced approximately 200-fold (5,6).

The application of these techniques to the task of isolating r-protein genes has in general proved remarkably successful. Of the fifty-two loci encoding r-proteins in E. coli all but eleven (possibly ten), have been obtained in the form of specialised λ transducing phages (Table 1). The magnitude of this sucess must however be attributed in part also to the clustered distribution of many r-protein genes in several areas of the chromosome. This clustering has allowed the fortuitous isolation of many r-protein genes within phages selected specifically, albeit indirectly, for others closely linked.

Table 1. Transducing phages containing ribosomal protein genes.

Transducing phage	Protein(locus)	Selected marker*	Reference
λdapB2	S20(rpsT)	dapB(p)	9
λpolC9	S2(rpsB) EFTs(tsf)	tonA(l) polC/dapD(p)	12
λpolC43		"	13
λserC	S1(rpsA)	serC(p)	10
λgrpE22	S16(rpsP) L19(rplS)	grpE(p)	14
λtyrA		tyrA(p)	14
λrpoD	σ(rpoD)+ possibly S20(rpsU)**	rpoD(p)	15
λfus2	S3(rpsC) L2(rplB) S4(rpsD) L3(rplC) S5(rpsE) L4(rplD) S7(rpsG) L5(rplE) S8(rpsH) L6(rplF) S10(rpsI) L14(rplN) S11(rpsK) L15(rplO) S12(rpsL) L16(rplP) S13(rpsM) L17(rplQ) S14(rpsN) L18(rplR) S17(rpsQ) L22(rplV) S19(rpsS) L23(rplW) L24(rplX) α(rpoA) L29(rpmC) EFG(fusA) L30(rpmD) EFTu(tufA)	aroE(p) trkA(p) fusA(p)	9,16
λpyrE	L28(rpmB) L33(rpmG)	pyrE(p)	11
λrif 18	L1(rplA) β(rpoB) L10(rplJ) β'(rpoC) L11(rplK) EFTu(tufB) L7/L12(rplL)	bfe(l) rif(p)	7,17,18

Table 1 (cont'd). Transducing phages containing ribosomal protein genes.

Transducing phage	Protein (locus)	Selected marker	Reference
λ*cycB*	S6 (*rpsF*) S18 (*rpsR*) L9 (*rplI*)	*cycB* (p)	11

* selection at the level of lysogen (l) on the basis of marker inactivation, or at the level of transducing phage (p) on the basis of marker complementation.

† hybrid phages constructed *in vitro*.

** based upon the recently reported map position of S21 (*rpsU*) (19).

Mapping of ribosomal protein genes

Two principal approaches have been used to map genes encoding r-proteins on the *E. coli* chromosome. The first, employing antibiotic-resistance and conditionally-lethal mutants with lesions in defined r-protein genes (20), has allowed mapping of selected loci in widely dispersed regions of the chromosome. The second, making use of transducing phages containing such regions and exploiting the clustered distribution of r-protein loci, has provided the locations of r-protein genes closely linked to those already mapped (Table 2). The benefit realised from the use of these complementary approaches has been considerable. In spite of the difficulties involved in mapping loci encoding essential cell components, the locations of all but four of the fifty-two genes for r-proteins have been established on the *E. coli* K12 linkage map.

Analysis of ribosomal protein gene organisation and fine structure

The availability of transducing phages containing r-protein genes has in several instances provided an ideal source of material for investigations of the organisation and fine structure of these loci. The variety of techniques used in these analyses is particularly well-illustrated

Table 2. Mapping of ribosomal protein genes.

Protein (locus)	Map position (min)	Reference
S1 (rpsA)	20	21
S2 (rpsB)	4	22
S3 (rpsC)*	72	9,16
S4 (rpsD)	72	23
S5 (rpsE)	72	24
S6 (rpsF)	94	25
S7 (rpsG)	72	26
S8 (rpsH)*	72	9,16
S9 (rpsI)	not known	-
S10 (rpsJ)*	72	9,16
S11 (rpsK)*	72	9,16
S12 (rpsL)	72	27
S13 (rpsM)*	72	9,16
S14 (rpsN)*	72	9,16
S15 (rpsO)	68	28
S16 (rpsP) (*)	56	14,29
S17 (rpsQ)	72	30
S18 (rpsR)	72	31
S19 (rpsS)*	72	9,16
S20 (rpsT)	0	32
S21 (rpsU)	66	19
L1 (rplA)*	88	17,18
L2 (rplB)*	72	9,16
L3 (rplC)*	72	9,16
L4 (rplD)	72	33
L5 (rplE)*	72	9,16
L6 (rplF)*	72	9,16
L9 (rplI)	94	34
L10 (rplJ)*	88	17,18
L11 (rplK)*	88	17,18
L7/L12 (rplL) (*)	88	17,18
L13 (rplM)	not known	-
L14 (rplN)*	72	9,16
L15 (rplO)*	72	9,16
L16 (rplP)*	72	9,16
L17 (rplQ)*	72	9,16
L18 (rplR)*	72	9,16
L19 (rplS)	56	35
L20 (rplT)	not known	-
L21 (rplU)	68	28
L22 (rplV)*	72	9,16
L23 (rplW)*	72	9,16
L24 (rplX)*	72	9,16

Table 2 (cont'd). Mapping of ribosomal protein genes.

Protein (locus)	Map position (min)	Reference
L25 (rplY)	47	36
L27 (rpmA)	68	28
L28 (rpmB)	83	37
L29 (rpmC) *	72	9,16
L30 (rpmD) *	72	9,16
L31 (rpmE)	89	38
L32 (rpmF)	30	cited in 20
L33 (rpmG)	83	37
L34 (rpmH)	not known	-

* indicates r-protein loci mapped by identification of products encoded by λ transducing phages.

(*) indicates r-protein loci mapped using λ phages confirmed with the aid of conventional techniques.

in studies of phages λ*fus*3 (16) and λ*rif*18 (17,18), which contain genes of the 72 and 88 minute chromosomal regions respectively.

Initially, the analysis of these phages was directed toward establishing the order and grouping of r-protein genes of each region. This aim was achieved by the use of essentially two approaches. First, the analysis of proteins synthesised in UVL irradiated cells, lysogenic and non-lysogenic, infected with the parental phages and their deleted derivatives (16,39-44). Second, the analysis of products synthesised in *in vitro* cell free systems primed with individual restriction fragments prepared from each phage (45-47). More recently, studies using the same transducing phages, or more precisely subclones containing restriction fragments derived from them, have provided a more detailed picture at the level of nucleotide sequence, of the fine structure of the transcriptional units in which r-protein genes of these regions are grouped (48-52).

These investigations have in combination allowed for a precise definition of features of the organisation of both r-protein gene clusters important for their regulated expression.

Studies of the regulation of ribosomal protein gene expression

The regulatory mechanisms ensuring the coordination of synthesis of individual r-proteins and collective sensitivity of these syntheses to conditions of cell growth have been the subject of intense research interest for a number of years (see 4). Specialised λ transducing phages containing r-protein genes have served in a variety of capacities in these studies.

The analysis of mechanisms underlying coordination of expression has involved two principal approaches. In the first, employing transducing phages and their derivatives both in the construction of merodiploids and in the role of mRNA hybridisation probes, the influence of gene copy number on the expression of specific r-protein genes at transcriptional and post-transcriptional levels has been analysed in vivo (53-56). In the second, using transducing phage DNAs as templates for transcription and translation in vitro, the effect of individual r-proteins upon expression of specific r-protein operons has been examined (57-61). The combination of these approaches has, by suggesting the existence of an assembly-conditioned feedback regulatory mechanism governing the synthesis of r-proteins (reviewed in 62), significantly enhanced our understanding of the means by which coordinated accumulation of ribosom-

al components might be accomplished.

The analysis of mechanisms underlying the effects of growth rate and amino acid starvation upon r-protein gene expression, which has involved a variety of approaches, has also relied heavily upon the use of transducing phages; as mRNA hybridisation probes (63-66), and as templates for transcription and translation in vitro (67-69).

Ribosomal protein S1

The physical properties of protein S1, a protein of the small ribosomal subunit so named because of its behaviour in the two dimensional polyacrylamide gel electrophoretic system of Kaltschmidt & Wittmann (see 70), have been the subject of much recent interest. Protein S1 differs from the majority of r-proteins in possessing a relatively high molecular weight ($M_r \sim 65,000$ compared to an average $M_r \sim 17,500$, 70), and relatively low pI (pI ~ 5.0 compared to an average basic pI, 71). One focus of this attention has been the observed ability of S1 to interact in a specific manner with nucleic acids (72,73). The characteristics revealed by the analysis of this interaction (72-77), have enhanced our understanding of the protein's function in vivo in two important regards. First, in respect of its requirement for translation, the ability of S1 to bind and melt double-stranded nucleic acids has indicated a role

for the protein in inducing melting of secondary structure at the 5' end of mRNA, as a prelude to translation initiation (77). Second, in regard to its participation in the replication of the single-stranded RNA phage Qβ (78), the specificity of this interaction for particular single-stranded RNA sequences has pointed toward a role for the protein in determining the specific binding of replicase to regions of the single-stranded template (79,77).

The regulation of the synthesis of protein S1 has been the subject of investigation also, but to a considerably lesser degree. The cellular level of S1 has been shown to be conditioned by growth rate (80) and responsive to nutritional enrichment (81), in the manner characteristic of an r-protein. However, the regulatory mechanisms ensuring this pattern of synthesis have remained to a large extent unexplored. Recent mapping of the gene encoding S1 (*rpsA*) (21), has made possible the isolation of a specialised transducing phage carrying this locus as a first step toward elucidation of the gene's organisation and fine structure. The study reported here, which describes the identification and characterisation of a transducing phage containing *rpsA*, was undertaken to provide an instrument for investigations of these and other aspects of the mechanisms underlying regulation of S1 synthesis.

METHODS I

Bacterial and bacteriophage strains

The E. coli K12 and λ phage strains used in this study are described according to derivation and relevant genotype in Table 3.

The transducing phage λaroA1338 (hereafter referred to as λaroA) was derived from the parent λcI857xis6S7nin5b515 b519 (hereafter referred to as λ helper) by an established procedure (5; R. Weisberg, personal communication).

Preparation of bacteriophages

λ helper phage

Crude lysates of λ helper were prepared by the plate lysis method (82) using the indicator strain RW262.

Strain RW262 was grown for this purpose in Tryptone broth (TB), and enriched medium containing per litre; 10 g Bactotryptone, 5 g NaCl supplemented with thiamine (1 μg/ml) and maltose (0.2%). Exponentially growing cultures of RW262 at A_{600} = 1.0 were removed, brought to 10 mM $MgSO_4$ and mixed with λ helper stock (multiplicity of infection 0.001) in TM buffer comprising; 10 mM tris.HCl (pH 7.4), 10 mM $MgSO_4$. After preadsorption for 10 min at 37°C,

Table 3. Relevant characteristics of bacterial and phage strains.

Strain	Relevant genotype and characteristics	Source
S159	uvrA,strA,sup0; UVL-sensitive (83)	J. Friesen
S159(λpapa)	S159 lysogenised with λpapa (84)	J. Friesen
RW262	supF,tonA; permissive for S7 mutants (5)	R. Weisberg
RW1315	aroA,strA,sup0:Tn10$_{130}$ requiring aromatic amino acids	R. Weisberg
RW1338	RW1315 lysogenised with both λcI857xis6S7nin5b515b519 and its derivative, λaroA1338, a defective transducing phage complementing the aroA$^-$ mutation	R. Weisberg
C600	F$^-$, (85)	B. Bachmann

0.3 ml aliquots of this mixture were added each to 3 ml TB 0.3% agar and immediately poured in overlay onto fresh TB 1.5% agar plates. The plated mixtures of phage and host were incubated at 37°C for 5-6 hr to confluent lysis, harvested and treated with 1% chloroform over 15 min at 4°C to complete the release of phages. Crude lysates of λ helper were then cleared of agar and cell debris by centrifugation firstly at 6,500 rpm/5 min/4°C then at 10,000 rpm/20 min/4°C (Sorvall; SS34).

Purified λ helper was prepared from crude lysates by a combination of differential and CsCl density gradient centrifugation. Phages were pelleted by centrifugation at 15,000 rpm/4 hr/4°C (Beckman; Type 30), and the pellets shaken dry then overlayed with small volumes of TM buffer. After gently mixing for 18-24 hr at 4°C, the resuspended material was pooled in 2 ml total volume and cleared of aggregates by centrifugation at 6,500 rpm/5 min/4°C (Sorvall; SS34). The supernatant was adjusted with saturated CsCl solution ρ = 1.52 g/ml and spun to equilibrium at 40,000 rpm/20 hr/4°C (Beckman; SW50.1). A band representing purified λ helper phage was then collected by lateral puncture in 1 ml and dialysed twice against 200 volumes of TM buffer.

The phage concentration in purified preparations produced from 20 plates of confluent lysate were usually of the order of 3.5×10^{11} plaque forming units/ml.

λ*aroA* transducing phage

Crude lysates of the defective transducing phage λ*aroA* were prepared by thermal induction of the lysogen RW1338. Strain RW1338 was grown for this purpose in M9G medium, an M9 minimal salts medium lacking aromatic amino acids but containing per litre; 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl, 1 g NH_4Cl, 0.25 g $MgSO_4.7H_2O$, 0.01 g $CaCl_2.2H_2O$ supplemented with thiamine (1 μg/ml) and glucose (0.4%), in order to select against the loss of this transducing phage reported to occur under less exacting nutritional conditions (R. Weisberg, personal communication).

Cultures (500 ml) grown in M9G medium at 30°C to A_{600} = 0.50, were heated rapidly to 42°C for 15 min then cooled and incubated at 37°C for 4 hr. Crude lysates of the lysis defective λ*aroA* were obtained from concentrated cell suspensions prepared at the end of this incubation by pelleting at 8,000 rpm/20 min/4°C (Sorvall; GSA) and resuspending in TM buffer (20 ml). Lysis of these cells was accomplished by the addition of chloroform (5%) and periodic agitation over 30 min at 4°C, and resulting phage preparations were cleared of cell debris and chloroform by centrifugation at 8,000 rpm/20-30 min/4°C (Sorvall; GSA).

Purified preparations of phage λ*aroA* were produced by differential then CsCl density gradient centrifugation of crude lysate material as with λ helper. In every instance this procedure yielded a single CsCl phage band correspon-

ding to the defective transducing phage, as anticipated from the reported poor efficiency of induction of λ helper from this strain (R. Weisberg, personal communication). Concentrations of phages in pure suspensions prepared in this way were generally of the order of 10^{12} phages/ml, as judged by comparison of the intensities of bands in CsCl of the transducing and a titratable phage.

UVL irradiation and infection of bacteria

The bacterial strains and procedures used in the UVL irradiation system were principally as developed by Ptashne (83). Strains S159 and S159(λpapa) were grown at 37°C in M9M medium (30 ml); M9 minimal salts medium supplemented with thiamine (1 μg/ml) and maltose (0.4%), in dual label experiments with the addition of L-((U)-^{14}C) lysine (338 mCi/mmol) at an early stage to prelabel. During the mid exponential phase, at A_{600} = 0.40, cultures of each strain were chilled and the cells concentrated by pelleting 10,000 rpm/10 min/4°C and resuspending at A_{600} = 2.0 in M9M medium containing 10 mM $MgSO_4$. A volume of each cell suspension (4 ml) was then transferred for irradiation into a glass petri dish, and exposed evenly to UV light (Ultraviolet Products Inc.; UVS54) to a dosage of 500 J/m^2. Immediately thereafter samples (300 μl) of the irradiated cultures were removed for incubation at 37°C for 15 min, with the simultaneous addition of TM buffer to

the uninfected samples, or of purified λ helper or transducing phage at a multiplicity of infection 5-10 (30 µl) to those intended for infection. At the end of this preadsorption period all cultures were diluted 5-fold with pre warmed M9M medium (1.2 ml) containing either L-(^{35}S) methionine (1013.9 Ci/mmol), or in dual label experiments L-(4,5-^{3}H(N)) lysine (78.1 Ci/mmol), and the incubation at 37°C continued for 30 min to pulse label. Incorporation of label was then terminated by chilling rapidly, followed immediately by the addition of a 100-fold excess of the appropriate non radioactive amino acid plus 10 mM sodium azide in a small volume (165 µl).

Samples (250 µl) taken at this stage for SDS polyacrylamide gel electrophoresis were quickly reacted with chilled 10% trichloroacetic acid (250 µl) over 1 hr at 0°C. The resultant precipitates were pelleted at 12,800 g/5 min/4°C, washed with ice-cold acetone (1 ml), then solubilised in SDS-sample buffer (100 µl) by heating to 100°C for 4 min. This material was stored at -20°C until required for analysis.

Determination of radioactivity incorporated into protein

Measurements of total incorporation of radioactivity into protein were made using a conventional filter disc method adapted from that developed by Mans & Novelli (86).

Samples (10 µl) were removed, dried onto filter discs (Whatman 934AH), and the discs then washed twice for 60 min at 0°C in 10% trichloroacetic acid containing a 1000-fold excess of the appropriate non radioactive amino acid. Each disc was rinsed with three changes of 5% trichloroacetic acid, first at 0°C for 5 min, then at 100°C for 30 min and finally again at 0°C for 5 min. After washing for 30 min at 42°C in two changes of ethanol, the discs were dried, transferred each to a vial containing a volume of Protosol (New England Nuclear) (1 ml), and digested at 45°C for 12 hr. Volumes of glacial acetic acid (50 µl) and toluene-Omnifluor liquid scintillant (New England Nuclear) (10 ml), were then added to each vial and the samples counted.

Immunoprecipitation of S1

Preparation of cell extracts

Extracts to be reacted with anti serum were prepared according to a modification of the method of Miyajima & Kaziro (80). Samples of each cell suspension (800 µl) were centrifuged at 12,800 g/10 min/4°C, the cell pellets resuspended thoroughly in 20 mM tris.HCl (pH 8.0) (80 µl) containing 25% sucrose, and a volume (16 µl) of a fresh 1:1 mixture of lysozyme (2.5 mg/ml in 0.1M tris.HCl, pH 8.0) plus 25 mM $(Na)_2$ EDTA (pH 8.0) added. After a 10

min incubation at 0°C, these suspensions were treated with a lysis solution (LS) (32 µl) containing; 2% NP-40, DNase (80 µg/ml) and RNase (400 µg/ml) in 40 mM $MgCl_2$, 0.8 M KCl for a further 15 min at 37°C. Cell lysates were then diluted 5-fold in NP40 buffer (512 µl) comprising; 25 mM potassium phosphate (pH 7.6), 0.1 M NaCl, 1 mM $(Na)_2$ EDTA and 0.5% NP-40, and cleared of cell debris by centrifugation at 27,000 rpm/30 min/4°C (Beckman; 75Ti). The resultant supernatants were stored at -70°C until needed.

Immunoprecipitation

Supernatants (200 µl) adjusted to 5 mg/ml BSA (20 µl), and dispensed in Eppendorf tubes were reacted with a volume of antiserum to S1 (20 µl) representing equivalence, during a 15 min incubation at 0°C. The reacted mixtures were then treated with a suspension of freshly washed protein A-sepharose CL-4B (Pharmacia) (5%) in NP40 buffer (150 µl) to adsorb the immune complexes, and incubated for a further 15 min at 0°C. Immunoprecipitates were pelleted by centrifugation at 12,800 g/60 sec at room temperature (Eppendorf microfuge), and washed six times in TSS buffer (1 ml) comprising; 50 mM potassium phosphate (pH 7.6), 0.1 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS. Following two washes in the same buffer lacking detergent (1 ml), each precipitate was resuspended in a volume of SDS-sample buffer (100 µl) containing; 62.5 mM tris.HCl

(pH 6.8), 1% glycerol, 5% 2-mercaptoethanol and 1.5 % SDS, and heated to 100°C for 4 min to solubilise the precipitated material.

For measurements of the total amount of radioactivity associated with each immunoprecipitate, the complete solubilised precipitates plus supernatants were transferred into Aquasol (New England Nuclear) (10 ml) to be counted.

Samples for analysis by SDS polyacrylamide gel electrophoresis were prepared from the supernatants remaining after pelleting of immunoadsorbent at 12,800 g/60 sec at room temperature.

SDS polyacrylamide gel electrophoresis of proteins

SDS polyacrylamide gel electrophoresis

The SDS polyacrylamide gel electrophoretic analysis of samples employed the discontinuous system developed by Laemmli (87) adapted to a vertical slab gel apparatus (Hoefer Scientific Instruments; SE500). The upper stacking component (10 x 0.75 mm) of each gel comprised invariably; 3% acrylamide (acrylamide:bisacrylamide, 36.5:1), 0.125 M tris.HCl (pH 6.8), 0.1% SDS, while the lower resolving component was made with either uniform or gradient concentrations of acrylamide giving the desired resolutive ranges of protein molecular size. Uniform resolving gels (90 x 0.75 mm) were prepared in the same way as the stacking gel, containing; 10% acrylamide (acrylamide:bisacrylamide, 36.5:

1), 0.375 M tris.HCl (pH 8.8), 0.1% SDS. Exponential gradient gels, with ranges of acrylamide concentrations of 10-20 or 25% acrylamide (acrylamide:bisacrylamide, 36.5:1), on the other hand were prepared by a somewhat different procedure; by mixing solutions of 10% and 20 or 25% acrylamide containing; 0.375 M tris.HCl (pH 8.8), 0.1% SDS, in the ratio 11:5 in a gradient mixer (Hoefer Scientific Instruments; Expo77). Both lower resolving and upper stacking gels were polymerised by the addition of TEMED plus ammonium persulphate.

Samples in SDS-sample buffer plus 0.01% bromophenol blue were loaded into wells in the stacking gel, and overlayed carefully with a tris-glycine electrode buffer (TG buffer) comprising; 0.025 M tris.HCl (pH 8.3), 0.192 M glycine and 0.1% SDS. This material was then electrophoresed at 7.5 mA to stack and 15-20 mA to achieve the desired resolution, as indicated by the migration of the tracking dye front.

Gels were fixed in 20 volumes of 12.5% trichloroacetic acid at 90°C for 15 min, stained for 4 hr in this solution made to 0.1% Coomassie brilliant blue G-250, destained in several changes of 7% acetic acid, then processed for autofluorography.

Autofluorography

The procedure of Bonner & Laskey (88) for scintillation

autofluorography of polyacrylamide gels was adopted without modification. After treatment, gels were dried and exposed to Kodak RP X-Omat film at -70°C for an appropriate period.

Two dimensional polyacrylamide gel electrophoresis of proteins

Preparation of cell lysates

Samples were prepared for two dimensional polyacrylamide gel electrophoretic analysis by the O'Farrell procedure (89). Small culture volumes (400 µl) were centrifuged at 12,800 g/10 min at room temperature, and the cell pellets were resuspended in a lysis buffer (50 µl) containing; 9.5 M urea, 2% NP-40, 2% ampholytes (comprising 1.6% pH range 5-7 and 0.4% pH range 3-10) and 5% 2-mercaptoethanol. These cell suspensions were then subjected to five cycles of rapid freezing and thawing in liquid N_2 and warm water, and the resultant extracts stored at -70°C until needed.

O'Farrell polyacrylamide gel electrophoresis

The procedures for two dimensional polyacrylamide gel electrophoresis, isoelectric focusing in the first dimension followed by SDS electrophoretic separation in the second, were essentially as described by O'Farrell (89).

The first dimension gels were made as a solution containing in 5 ml; 2.75 g urea, 4% acrylamide (acrylamide:

bisacrylamide, 17.5:1), 2% NP-40, and 2% ampholytes (Pharmacia)(1.6% pH 5-7, 0.4% pH 3-10), and polymerised (110 x 2.5 mm) in glass tubes under a layer of 8 M urea, with the aid of TEMED plus ammonium persulphate. Before the run, gels covered with fresh overlays of lysis buffer (20 µl) were prerun according to a set regimen; 200 V/15 min, 300 V/30 min and 400 V/30 min, with 0.02 M NaOH cathode and 0.01 M H_3PO_4 anode solutions. Samples (20 µl) were then loaded onto these prerun gels, overlayed carefully with cathode solution and electrophoresed at 400 V/12 hr then 500 V/1 hr to focus. Following the run, gels were removed from their tubes into SDS-sample buffer (10 ml), and gently shaken for 1 hr at room temperature to equilibrate.

The second dimension gels were prepared in a vertical slab gel apparatus (SE500) by the method of Laemmli (87) (see SDS polyacrylamide gel electrophoresis). Each comprised a lower resolving gel (100 x 0.75 mm) of a uniform 10% acrylamide, and an upper 3% acrylamide stacking gel (25 x 0.75 mm), polymerised again with TEMED plus ammonium persulphate. Equilibrated first dimension gels were transferred each onto a stacking gel, fixed there with a gel sticker solution comprising SDS-sample buffer plus 0.001% bromophenol blue made to 1% agarose, and the SDS dimension run with TG electrode buffer at 7.5 mA to stack and 15-20 mA to resolve, until the tracking dye front approached the lower end of the resolving gel.

Gels were stained then processed for autofluorography (see SDS polyacrylamide gel electrophoresis of proteins).

METHODS II

Preparation of bacteriophage DNA

Purified transducing and λ helper phages were prepared from crude lysates as above (see Preparation of bacteriophages), with the modification that pelleted phages resuspended in TM buffer were first treated with DNase I (2 μg/ml) at 37°C for 30 min before clearing of aggregated material. After banding in CsCl density gradients, phages were collected in 1 ml then dialysed twice against 200 volumes of TEN buffer containing; 10 mM tris.HCl (pH 8.0), 50 mM NaCl and 1 mM $(Na)_2$ EDTA.

DNA was prepared from the CsCl banded phages by a method adapted from that of Kaiser & Hogness (90). Phage suspensions were passaged through three successive extractions with phenol previously equilibrated with 0.1 M tris.HCl (pH 8.0). Each cycle involved vigorous mixing at room temperature, first for 30 then 15 and finally 5 min, of equal volumes of suspension and phenol, separation of the mixture into aqueous and phenol phases by centrifugation at 5,000 rpm/5 min/20°C followed by removal of each phenol layer. One-tenth volume of 2 M sodium acetate (pH 5.6) was added to each extracted aqueous phase, and DNA precipitated over 12 hr at -20°C by the addition of two volumes of

prechilled ethanol.

Ethanol precipitated DNA was pelleted at 8,000 rpm/20 min/-20°C (Sorvall; HB4), gently washed with 70% ethanol prechilled to -20°C, and after draining well, redissolved in a small volume (100-200 µl) of TEN buffer for storage at -20°C until needed.

Preparations of phage containing 10^{12} plaque forming units generally yielded 25-50 µg DNA by this method, as judged by measurement of A_{260} and the relation;

$$1\ A_{260}\ \text{unit} = 50\ \mu g/ml$$

Restriction endonuclease digestion of DNA

Phage DNA was digested for analysis with a range of restriction enzymes, including; Ava I, Eco RI, Hin dIII, Pvu I, Sal I, Sma I (New England Biolabs); Bam HI, Sst I (Bethesda Research Labs); Bgl II, Hpa I (Boehringer-Mannheim). All reactions were performed in small volumes (20-40 µl), incubated at 37°C for 2-4 hr with 4-10 x excess of enzyme, to ensure complete reaction. Digestions with single enzymes were carried out in the assay buffer specified by the manufacturer, while double digestions were performed either simultaneously in mutually compatible conditions or sequentially with adjustments to reaction conditions between digestions. At the end of each incubat-

ion, samples were chilled and reactions halted by the addition of a volume of solution (5 µl) containing; 5 mM tris.HCl (pH 7.0), 50% glycerol, 5 mM $(Na)_2$EDTA, 0.125% bromophenol blue, which facilitated both sample loading and tracking during subsequent electrophoretic analysis.

Agarose gel electrophoresis of DNA

Electrophoretic analysis of restriction endonuclease digested phage DNA was performed in 0.8% agarose horizontal gels (Bethesda Research Labs; H0). The gel and tris.acetate (TA) electrode buffer contained; 40 mM tris.acetate (pH 8.2), 5 mM sodium acetate, 1 mM $(Na)_2$EDTA, supplemented with 0.5 µg/ml ethidium bromide to stain the DNA fragments. Samples were loaded carefully into wells set into each gel, beneath a covering layer of electrode buffer, and electrophoresed at between 3-4 V/cm to the desired extent as indicated by migration of the tracking dye. Gels were then removed to be photographed through a Wratten 23A filter using Polaroid film and UV illumination.

Generally, 0.4-0.5 µg DNA per well proved sufficient for the visualisation of fragments representing as little as 0.01 lambda genome equivalents.

Hybridisation analysis of bacterial DNA sequences contained by λ*aroA*

Preparation of *E. coli* C600 DNA

High molecular weight *E. coli* K12 (C600) DNA was prepared using a modification of the methods of Marmur (91) and Saito & Miura (92).

A culture of *E. coli* C600 grown to an A_{600} = 0.80 in Luria broth (1 litre) was harvested by pelleting at 5,000 rpm/20 min/4°C (Sorvall; GSA), and the cells frozen rapidly in liquid N_2 then transferred in 50 mM tris.HCl (pH 8.0) (10 ml) containing sucrose and 1 mM $(Na)_2$EDTA, and chilled. Volumes of 0.25 M $(Na)_2$EDTA (pH 7.0) (2 ml), fresh lysozyme (5 mg/ml in 25 mM tris.HCl, pH 8.0) (1 ml) and RNase (10 mg/ml in 0.1 M sodium acetate, pH 7.0) (0.1 ml), pretreated at 80°C for 10 min to inactivate DNase, were added sequentially to each suspension. The cells, reduced to sphaeroplasts by incubation in this condition at 0°C for 15 min, were then treated with a lytic mixture (5 ml) containing; 0.3% Triton X-100 in 0.18 M $(Na)_2$EDTA; 0.15 M tris.HCl (pH 8.0), over a second period of 15 min at 0°C to promote lysis.

Each cell lysate diluted 1:1 with water (14 ml), was extracted 15 min at 0°C with a two-third volume of cold phenol previously equilibrated against 50 mM tris.HCl (pH 7.5), 0.1 M NaCl. After separating the phenol and aqueous

phases by centrifugation at 6,500 rpm/10 min/4°C (Sorvall; HB4), the extraction was repeated in the presence of a volume of chloroform equal to that of phenol. Following a second centrifugation at 6,500 rpm/15 min/4°C, the upper aqueous phase (15 ml) was removed, re-extracted with an equal volume of phenol by mixing at room temperature for 5 min, and centrifuged again at 6,500 rpm/10 min/room temperature. The aqueous phase (14 ml) containing chromosomal DNA was then adjusted to 0.2 M NaCl by the addition of 5 M NaCl (560 µl), and the DNA precipitated with two volumes of prechilled ethanol at -20°C overnight.

The DNA was pelleted by centrifugation at 6,500 rpm/60 min/20°C, redissolved in a large volume of TEN buffer (5 ml), and as a final stage in the purification, freed from contaminating oligonucleotides by precipitating a second time with one and one half volumes of ethanol for 30 min at room temperature. After pelleting at 12,800 g/5 min/ room temperature in an Eppendorf microfuge, the precipitate was dissolved in TEN buffer (100 µl).

The yield of DNA obtained using this procedure was approximately 140 µg from 1 litre of cell suspension. A sample (1 µg) of this purified preparation when analysed by agarose gel electrophoresis produced a single high molecular weight band, without detectable contamination.

Southern blot

The method used for transfer of restriction endonuclease generated DNA fragments separated by agarose gel electrophoresis onto nitrocellulose was essentially as developed by Wahl (93). The transducing and λ helper phage DNAs and E. coli C600 chromosomal DNA were each digested singly with enzymes Pvu I and Hpa I under the conditions specified by the supplier. Samples of these digestions amounting to 2.5 μg of C600 bacterial and 25 ng of each phage DNA were then subjected to electrophoretic separation in a 0.8% agarose gel of 8 mm thickness, using the TA buffer system. After electrophoresis at 4 V/cm for approximately 5 hr in the presence of ethidium bromide, the gel was removed to be photographed and then processed before blotting. This processing, which involved sequential washing of the gel first with two changes of 0.25 M HCl (250 ml) for 15 min each, then after a brief rinse with distilled water with two changes of 0.5 M NaOH, 1 M NaCl (250 ml) for 15 min, made for uniformly efficient transfer of denatured restriction fragments across a wide size range. Following the final treatment with alkali, the gel was adjusted to neutral pH by washing with two changes in 0.5 M tris.HCl (pH 7.4), 3 M NaCl (250 ml) for 15 then 30 min, and placed in the blotting apparatus. For transfer, the gel was positioned on a platform, framed with Saranwrap and sandwiched in intimate contact between three sheets of

Whatman 3MM paper saturated and dipping into a reservoir solution of 20 x SSC comprising 3 M NaCl, 0.3 M trisodium citrate (below), and a sheet of nitrocellulose (Schleicher & Schuell; BA85, 0.45 μM) wetted with distilled water (above). Three additional sheets of dry Whatman paper were then placed in uninterrupted contact over the top of the nitrocellulose, and a 5 cm layer of paper towels supporting a light weight to ensure even contact added to complete the apparatus.

After the transfer had been allowed to proceed for 36 hr, the nitrocellulose sheet was removed for baking at 80°C for 2 hr under vacuum, as a final step before prehybridisation.

At this point restaining the gel with ethidium bromide in TA buffer followed by examination under UV light confirmed that no DNA remained in the gel.

In vitro (^{32}P) radioactive labeling of λaroA DNA

(^{32}P) labeling of transducing phage DNA by nick translation was performed using a system supplied by New England Nuclear, according to a procedure essentially as described by the manufacturer. Reaction mixtures (30 μl) each contained; 25 μCi dCTP (γ-^{32}P) (3400 Ci/mmol), 0.4 nmoles each of non radioactive dATP, dGTP and dTTP, 1.2 units of DNA polymerase I, 50 pg DNase I (Worthington Biochemicals) and 0.5 μg purified λaroA DNA in a buffer supplied by the

manufacturer. DNase I for the reactions was prepared by the method of Rigby (94), involving activation for 2 hr at 0°C in a buffer comprising; 10 mM tris.HCl (pH 7.5), 5 mM $MgCl_2$, 1 mg/ml BSA. Labeling commencing with the addition of DNase I was performed at 13°C for 3 hr than halted by adding 0.25 M $(Na)_2$EDTA (pH 8.0) (10 µl) and heating to 70°C for 3 min. The products of the reaction were immediately diluted 5-fold in TEN buffer and applied in a minimum volume to a column of Sephadex (fine) G-50 (Pharmacia) previously equilibrated and run with the same buffer. The nick-translated DNA which elutes in the void volume, was then collected in 1 ml and stored at 4°C until required for hybridisation.

For measurement of the specific activity of the probe, samples (2 x 1 µl) of both the stopped reaction and the column eluate were spotted onto filter discs with 5 µg carrier calf thymus DNA, then precipitated and washed with chilled 10% trichloroacetic acid then ethanol, and counted in 10 ml toluene-Omnifluor. In these analyses, levels of incorporation of radioactivity 40-60% of total, representing specific activities of 5-7 x 10^7 cpm/µg λ*aroA* DNA, and recoveries of incorporated label from the column of 90-100%, were generally achieved.

Hybridisation

Southern blots prepared according to the procedures

described were further processed along the lines set out by Wahl (93). In the prehybridisation step, each paper was sealed in a polyethylene bag with a volume of prehybridisation mix (10 ml) containing; 50% deionised formamide (99% A.C.S. grade, BDH), 5 x SSC, 5 x Denhardt's reagent (comprising 0.02% each of BSA, polyvinyl pyrrolidone and Ficoll M_r 400,000), 1% glycine, 250 μg/ml freshly denatured, sonicated herring sperm DNA (Boehringer-Mannheim) and 50 mM sodium phosphate (pH 6.5), and incubated at 42°C for 10 hr. The prehybridised blots were removed and each placed in a second polyethylene bag with a part volume (9 ml) of a hybridisation mix (total volume 10 ml) containing; 50% deionised formamide, 5 x SSC, 1 x Denhardt's reagent, 10% sodium dextran sulphate (M_r 500,000) (Pharmacia) and 20 mM sodium phosphate (pH 6.5), while the remainder of the mix was heated to 65°C. The nick-translated probes (1.2 ml) each mixed with a small volume (10 μl) of sonicated herring sperm DNA (10 mg/ml) were denatured by heating at 100°C for 5 min, and cooled quickly in an ethanol-ice bath. The heated aliquot of hybridisation mix (1 ml) was then added to this freshly-denatured DNA, and the total mixed swiftly and thoroughly with the contents of the bag before sealing.

After incubating at 42°C for 20 hr, the hybridised blot was removed from the bag and washed three times in 2 x SSC, 0.1% SDS (250 ml) at room temperature for 5 min each, then

a further three times in 0.1 x SSC, 0.1% SDS (250 ml) at 50°C for 15 min each. This treatment reduced free radioactivity, estimated by Cerenkov counting 10 ml volumes of each successive wash, almost to background. The washed, hybridised blots were then exposed to Kodak RP X-Omat film at -70°C through a film of Saranwrap, for appropriate lengths of time.

RESULTS I

The studies described in this section were designed to determine whether a transducing phage, isolated on the basis of *aroA*⁻ complementation by R. Weisberg (5; R. Weisberg, personal communication), might carry the gene for r-protein S1 (*rpsA*) which is closely linked to *aroA* on the *E. coli* chromosome (21).

Immunoprecipitation of S1 from extracts of cells infected with a λ*aroA* phage

Preliminary screening of the λ*aroA* phage for an S1 coding capacity was accomplished by analysis of anti S1 reactive material synthesised in UVL irradiated cells of strain S159 so-infected. The results of this analysis, shown in Table 4, demonstrate that infection of S159 with λ*aroA* or with its parent phage λ helper, produced a stimulation in the synthesis of anti S1 reactive material of 21.4 and 2.5-fold respectively over the level found in uninfected cells. The stimulation represents more than 6% of total radioactivity incorporated in the λ*aroA*-infected cells.

Table 4. Immunoprecipitation of material from extracts of UVL irradiated S159 infected with λ*aroA*.

Source of extract	Immunoprecipitate cpm (^3H and ^{14}C)		Immunoprecipitate isotope ratio ($^3H/^{14}C$)	Normalised isotope ratio
S159 uninfected	^3H	1010	3.7	1.0
	^{14}C	270		
S159. λ helper	^3H	2940	9.5	2.5
	^{14}C	310		
S159. λ*aroA*	^3H	26400	80	21
	^{14}C	330		

A culture of strain S159 grown in the presence of L-((U)-^{14}C) lysine at 0.2 μCi/ml for at least three generations, was harvested, resuspended in minimal medium and irradiated with UVL. Volumes (300 μl) of the irradiated culture were preadsorbed with phage (30 μl) then diluted 5-fold in medium containing L-(4,5-^3H(N)) lysine at 100 μCi/ml, and incubated for 30 min. Samples (800 μl) taken from each of these subcultures were extracted, S30 fractions prepared (640 μl), and portions (200 μl) reacted with equivalence amounts of antibodies to S1 then of protein A-sepharose adsorbent. Washed immunoprecipitates solubilised in SDS-sample buffer (100 μl), were then transferred into 10 ml Aquasol for counting (all procedures as described in Methods).

The results thus obtained for the incorporation of radioactivity into immunoprecipitated material as presented, are in the form of (^3H)/(^{14}C) cpm ratios normalised to the value obtained with uninfected cells.

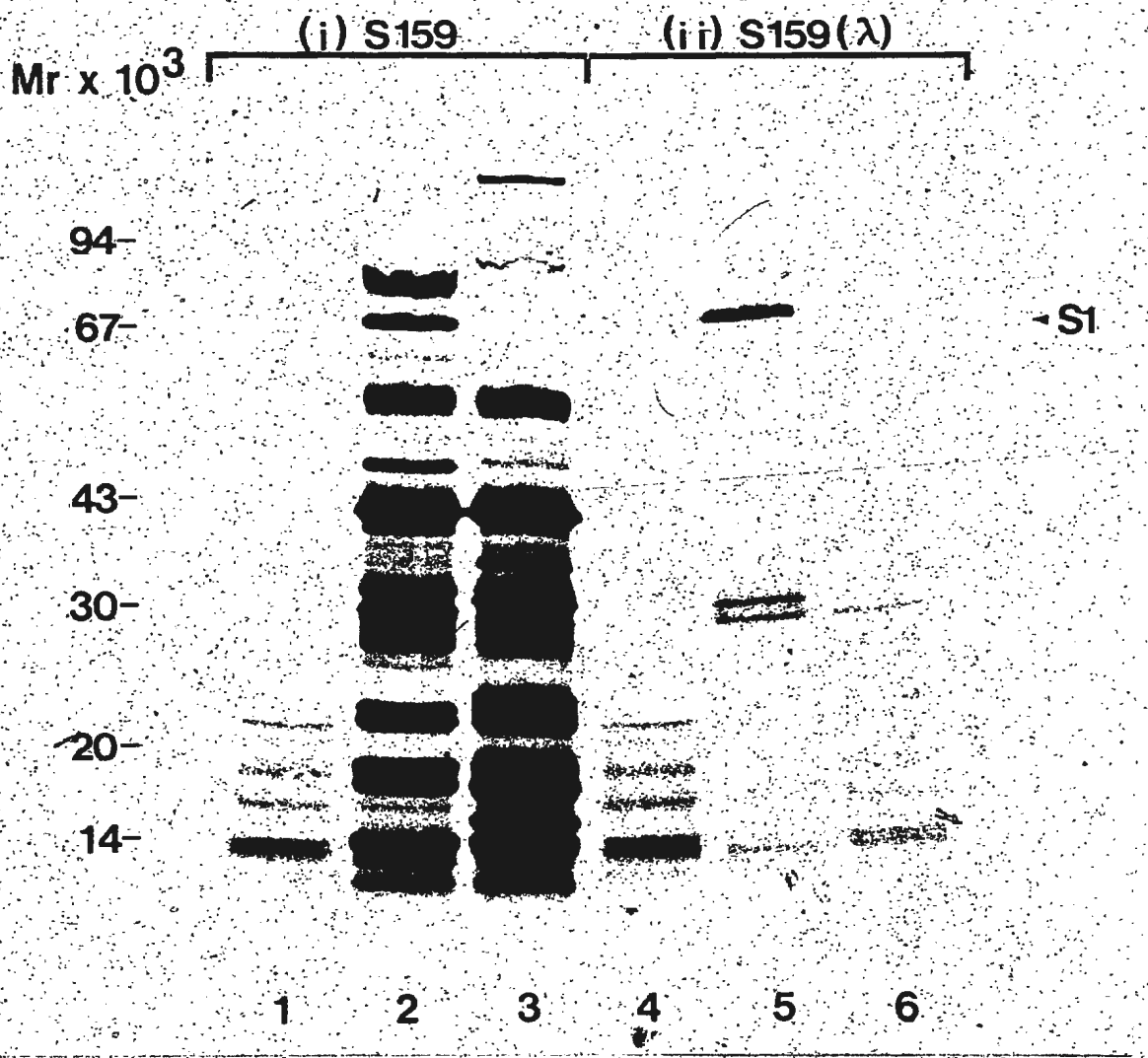
SDS polyacrylamide gel electrophoretic analysis of total material synthesised in λ*aroA*-infected cells

The enhanced synthesis of anti S1 reactive material observed in UVL irradiated cells infected with λ*aroA*, prompted a more detailed analysis of the proteins encoded by this phage. The form of this analysis was chosen to provide information both of the pattern of proteins coded for by λ*aroA* and the source, phage and bacterial, of promoter activities governing their expression. Using the UVL irradiation system, information of this nature was obtained by the use of alternative non-lysogenic and lysogenic hosts for infection with transducing phages. Proteins synthesised in irradiated cells of either type infected with λ*aroA* or parental λ helper were labeled with L-(^{35}S) methionine (labeling with L-((U)-^{14}C) lysine produced essentially identical results), extracted then analysed by SDS polyacrylamide gel electrophoresis.

The first part of this analysis (Figure 1 (i)), in which the host used was the non-lysogen S159, provides a comprehensive picture of the proteins encoded λ*aroA*. A comparison of the pattern of radioactively labeled products synthesised in this host infected with the transducing phage and its parent λ helper (lanes 2 & 3), reveals several differences between the coding capacities of the parental and derived phages. These differences presumably

Figure 1. SDS polyacrylamide gel electrophoresis of total proteins synthesised in UVL irradiated S159 and S159(λpapa) infected with λ*aroA*.

Cultures of strains S159 and S159(λpapa) grown in minimal medium were harvested, resuspended in medium containing 10 mM $MgSO_4$ then irradiated with UVL. Volumes (300 µl) of the irradiated culture were preadsorbed with phage (30 µl) then diluted 5-fold in medium containing L-(^{35}S) methionine at 100 µCi/ml, and incubated for 30 min. Samples (200 µl) taken from each of these subcultures were precipitated with trichloroacetic acid, solubilised in SDS-sample buffer (100 µl), and portions withdrawn for analysis alongside a pure S1 marker on an SDS 10-20% acrylamide gradient gel. After electrophoresis at 15 mA to stack and 30 mA to run (until 30 min after loss of the tracking dye front), the gel was stained and processed for autofluorography (all procedures as described in Methods). (Film exposure 7 days). Molecular weights were determined using Pharmacia LMW protein markers; phosphorylase b (M_r = 94,000), albumin (M_r = 67,000), ovalbumin (M_r = 43,000), carbonic anhydrase (M_r = 30,000), trypsin inhibitor (M_r = 20,000) and α-lactalbumin (M_r = 14,400). Lane 1 (16,200 cpm loaded) uninfected S159; lane 2 (58,950 cpm) S159.λ*aroA*; lane 3 (76,200 cpm) S159.λhelper; lane 4 (17,400 cpm) uninfected S159(λpapa); lane 5 (18,500 cpm) S159(λpapa).λ*aroA*; lane 6 (16,800 cpm) S159(λpapa).λhelper.


(i) S159
(ii) S159(λ)
Mr x 10^3
94–
67–
43–
30–
20–
14–
S1
1
2
3
4
5
6

reflect the substitution of bacterial for phage sequences which occurred in the creation of λ*aroA* from its parent. Proteins synthesised in λ helper and not λ*aroA*-infected cells can be ascribed to those phage sequences lost in the substitution, whose loss accounts for the defective character of λ*aroA*. While those six proteins, with M_r = 29,500; 38,000; 45,500; 64,000; 70,000 and 80,000, whose production is unique to the transducing phage-infected condition may be attributed to bacterial sequences acquired by λ*aroA* in place of those lost.

The second part of this analysis (Figure 1 (ii)), in which the lysogen S159(λpapa) was used as host, sheds some light on the likely origins, phage and bacterial, of the promoter activities governing the expression of bacterial genes carried by the transducing phage. The efficiency with which phage gene expression is repressed in the lysogenic host can be clearly appreciated from a comparison of proteins synthesised in the different hosts infected with λ helper; S159 (lane 3) and S159(λpapa) (lane 6). This repression can be seen to extend also to the production of phage proteins in S159(λpapa) infected with λ*aroA* (lane 5). In the λ*aroA*-infected lysogen only six radioactively labeled proteins are synthesised to any appreciable extent. One of these products (M_r = 27,000), also synthesised in helper-infected cells (lane 6), is probably the λcI repressor protein which is known to be produced under these

conditions (39). The remaining five, which comigrate with products shown in Figure 1 (i) to be unique to transducing phage infection of the non-lysogen, on the other hand probably represent bacterial proteins encoded by λ*aroA* which are transcribed from promoters of bacterial rather that phage derivation. The product of M_r = 45,500 is the only species of the six unique to λ*aroA* infection which appears to lack a bacterial promoter by this criterion.

A comparison of the migration of proteins synthesised in λ*aroA*-infected cells with the position of pure S1 marker run in the same analysis suggests the identification of one of the bacterial proteins coded for by this phage as the product of the *rpsA* gene. This protein possesses an M_r = 70,000, close to that reported for r-protein S1 in similar SDS polyacrylamide gel electrophoretic analyses (71).

SDS polyacrylamide gel electrophoretic analysis of material immunoprecipitated from extracts of λ*aroA*-infected cells

The relationship between bacterial coding capacities attributed to the transducing phage and the earlier observed enhanced synthesis of anti S1 reactive material in λ*aroA*-infected cells was explored further. The approach chosen to clarify this relation, once again making use of the UVL irradiation system, took the form of an electrophoretic analysis of species precipitated in reaction with

anti S1 serum. Proteins synthesised in irradiated cells of both S159 and S159(λpapa) infected with λ*aroA* or helper, were extracted, reacted with immune serum and the immunoprecipitated material analysed by SDS polyacrylamide gel electrophoresis.

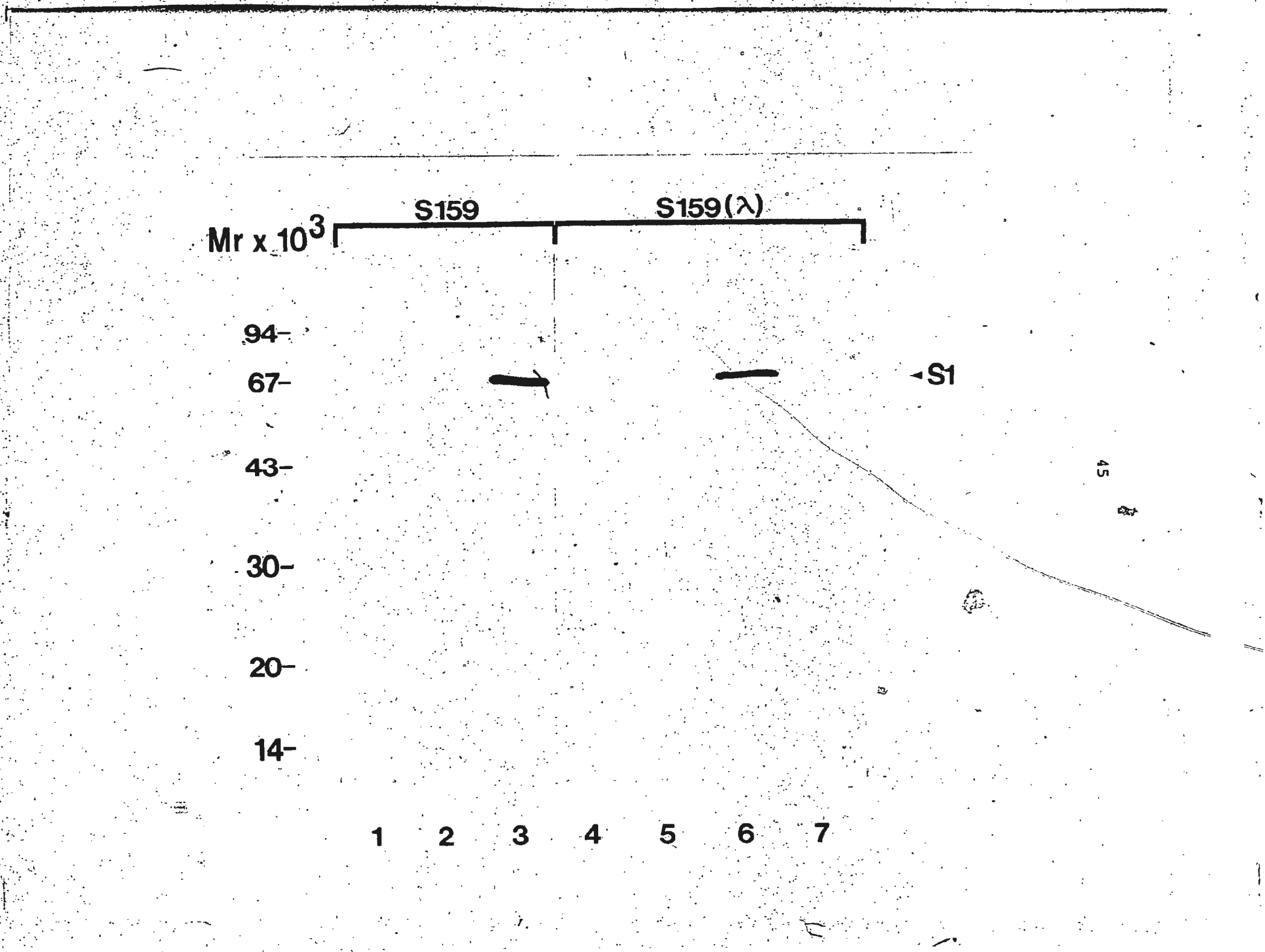
The first part of this analysis, presented in Figure 2 (i) shows clearly that an immunoreactive species, which is detected only in extracts of λ*aroA*-infected S159 (lane 3) and thus likely of bacterial origin, comigrates with pure S1. A second protein of M_r = 38,000 is precipitated from this extract but would seem, from its appearance in the immunoprecipitate produced from λ helper-infected cells, likely a phage protein (possibly the major capsid protein, gene E product).

The second part of the analysis (Figure 2 (ii)) establishes, in agreement with the results of Figure 1 (ii), that the S1 comigrating material is synthesised both in the non-lysogenic and (albeit at a slightly reduced level) the lysogenic host infected with λ*aroA* (lane 6), confirming its immunity from cI repression of phage gene expression. The protein of M_r = 38,000 referred to above is not detected in extracts of either λ*aroA* or λ helper-infected lysogen (lanes 6 & 5), as would be expected if it were a phage gene product.

The suggested immunological identity of protein S1 and the comigrating material synthesised in λ*aroA*-infected

Figure 2. SDS polyacrylamide gel electrophoresis of material immunoprecipitated from extracts of UVL irradiated S159 and S159(λpapa) infected with λ*aroA*.

Samples (800 µl) of cultures of strains S159 and S159.(λpapa) UVL irradiated and labeled with L-(^{35}S) methionine as specified in the legend to Figure 1, were extracted and S30 fractions (640 µl) prepared. Portions of these extracts were reacted with equivalence amounts first of antibodies to S1 then of protein A-sepharose adsorbent, and the resulting immunoprecipitates washed then solubilised in SDS-sample buffer (100 µl). Volumes (25 µl) of each of the solubilised samples were withdrawn for analysis alongside a pure S1 marker on an SDS 10% acrylamide gel. Following electrophoresis at 15 mA to stack and 30 mA to run (until the tracking dye approached 1 cm from the lower end), the gel was stained and processed for autofluorography (all procedures as described in Methods). (Film exposure 48 hr). Molecular weights were determined as in Figure 1. Lane 1 (120 cpm loaded) uninfected S159; lane 2 (450 cpm) S159.λ helper; lane 3 (3,760 cpm) S159.λ*aroA*; lane 4 (100 cpm) uninfected S159(λpapa); lane 5 (120 cpm) S159(λpapa).λ helper; lane 6 (1,900 cpm) S159(λpapa).λ*aroA*; lane 7 (60 cpm) as sample 6 except that the immune serum used for reaction with S159(λpapa).λ*aroA* extract was first incubated with an excess of pure S1 for 15 min at 0°C.


S159
S159(λ)
Mr x 10^3
94-
67-
43-
30-
20-
14-
S1
1
2
3
4
5
6
7

cells is further reinforced in Figure 2 (lane 7), by the demonstration that preadsorption of the anti serum with an excess of pure S1 before reaction with an extract of λ*aroA* infected S159(λpapa) results in complete elimination of this radioactively labeled protein from the immunoprecipitate.

Two dimensional polyacrylamide gel electrophoretic analysis of total material synthesised in λ*aroA*-infected cells

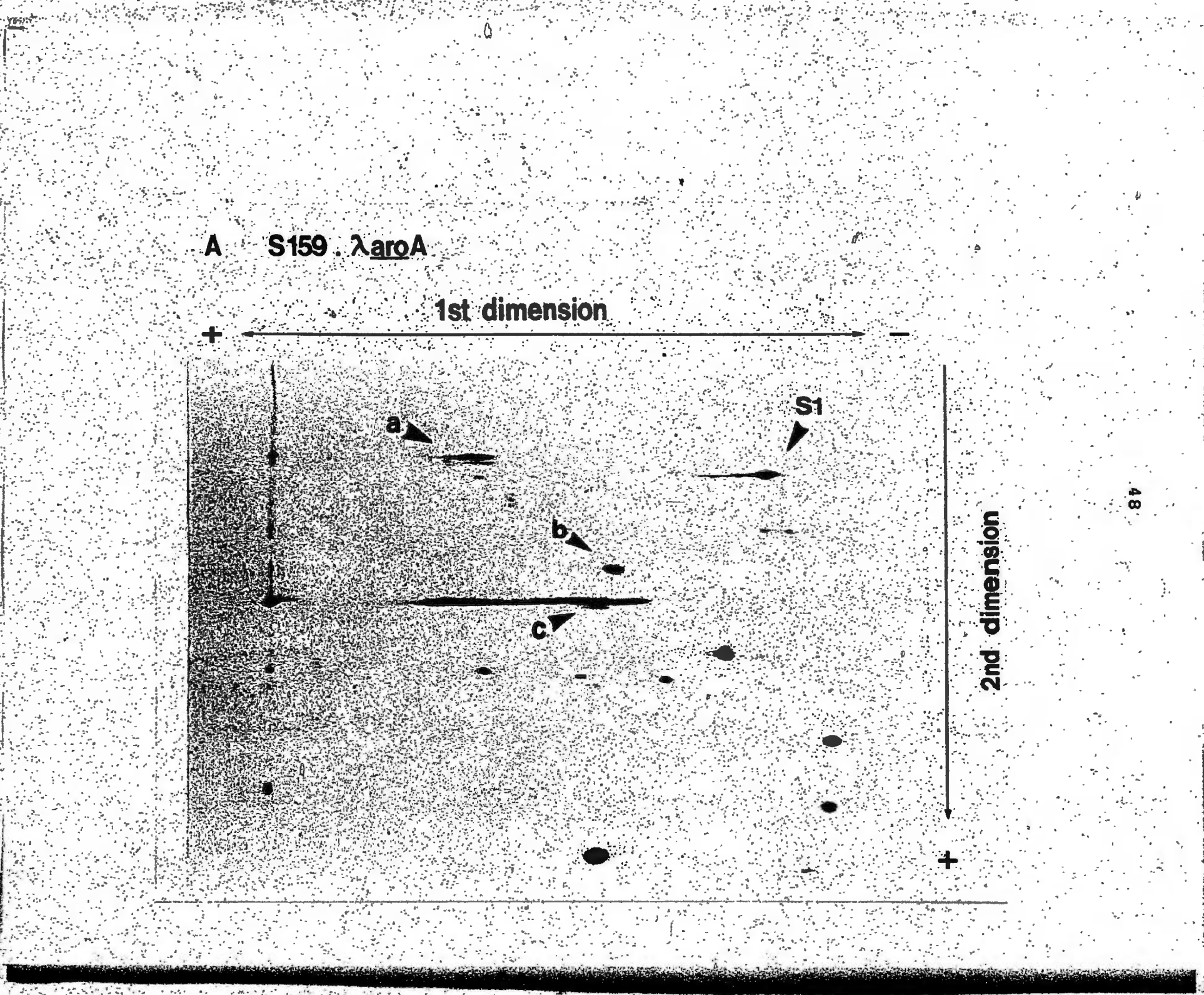
The nature of the bacterial proteins encoded by λ*aroA* was examined further using the UVL irradiation system this time coupled to the method for two dimensional polyacrylamide gel electrophoretic analysis devised by O'Farrell (89). Proteins synthesised in irradiated cells, both lysogenic and non-lysogenic, infected with this phage and lebeled with L-(^{35}S) methionine, were extracted then analsed using the O'Farrell technique.

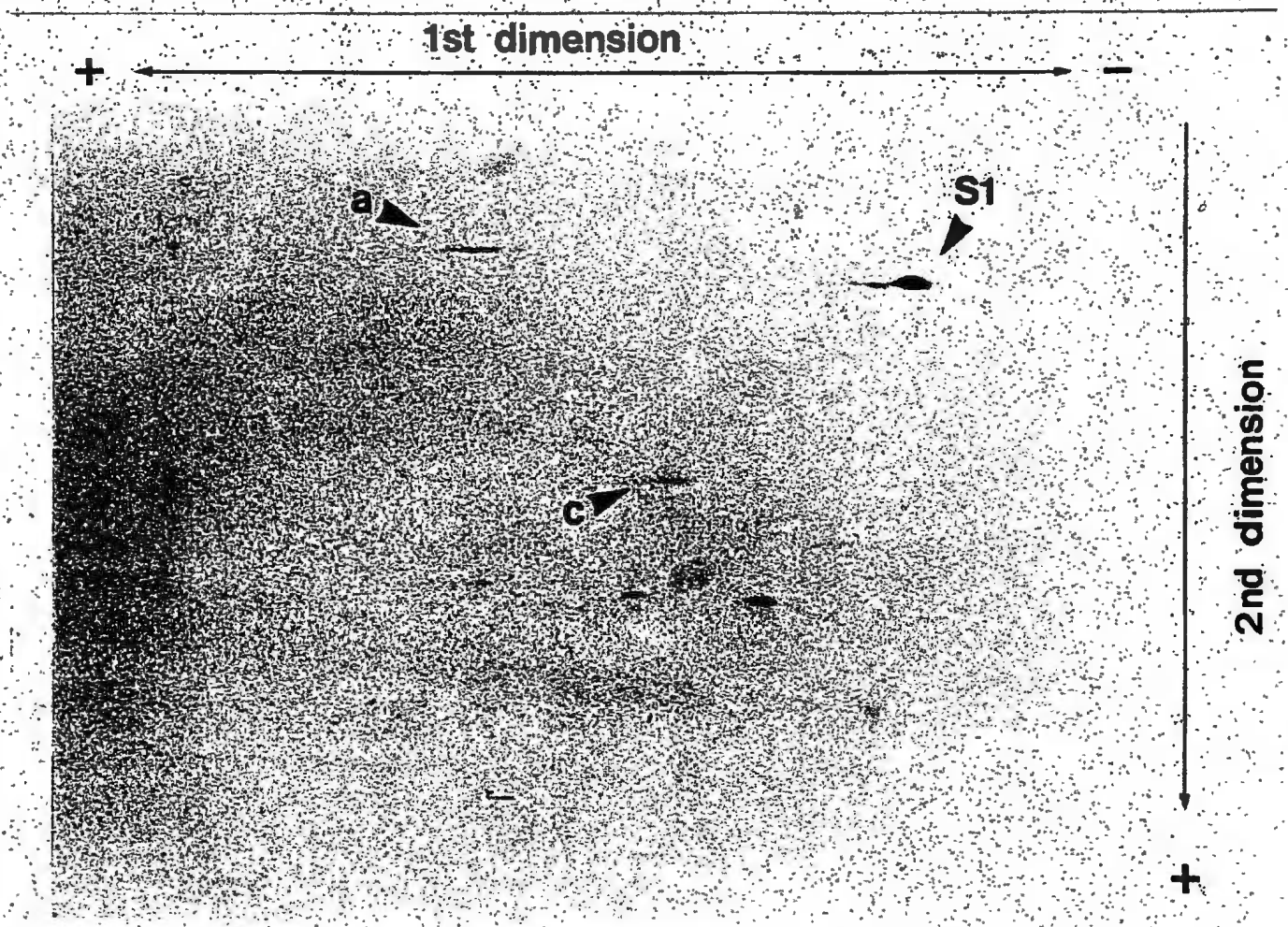
The analysis, shown in Figure 3 (a) (non-lysogen) and (b) (lysogen), clearly demonstrates that a bacterial protein synthesised in both types of host infected with this phage, and thus likely expressed from a bacterial promoter, comigrates with S1. This protein, which by such comigration is shown to be identical to S1 according to criteria both of isoelectric point and M_r behaves as expected in this gel system, migrating with a pI 5-6 and M_r = 70,000 (95).

The results of Figure 3 (a) and (b) in addition, provi-

Figure 3. Two dimensional polyacrylamide gel electrophoresis of total proteins synthesised in UVL irradiated S159 and S159(λpapa) infected with λ*aroA*.

Samples (400 μl) of cultures of strains S159 and S159 (λpapa) UVL irradiated and labeled with L-(^{35}S) methionine as specified in the legend to Figure 1, were lysed in small volumes (50 μl) of lysis buffer by freeze-thaw treatment. A portion (5 μl) of each lysate to which a quantity of pure S1 marker (1.1 μg in 25 μl of lysis buffer) had been added, was then withdrawn and analysed by the O'Farrell procedure. After electrophoresis for 5,300 V/hr in the isoelectric focusing first dimension, followed by 15 mA to stack and 30 mA to run (until the tracking dye approached 1 cm from the lower end) in the SDS second dimension, these gels were stained and processed for autofluorography (all procedures as described in Methods). Part (a) S159. λ*aroA* (81,400 cpm) (film exposure 36 hr); part (b) S159 (λpapa).λ*aroA* (17,100 cpm) (film exposure 66 hr).


A S159 . λaroA
1st dimension
+
−
S1
a
b
c
2nd dimension
+
−



B S159(λ) . λ<u>aroA</u>

de a more complete characterisation, in terms of pI and M_r, of several other of the bacterial proteins encoded by phage λ*aroA*.

RESULTS II

The experiments described in this section were aimed at establishing a restriction map for a section of the bacterial chromosome carried by the λ*aroA* transducing phage shown in previous analyses to contain the gene for r-protein S1 (*rpsA*).

Electrophoretic analysis of restriction endonuclease digests of λ*aroA* DNA

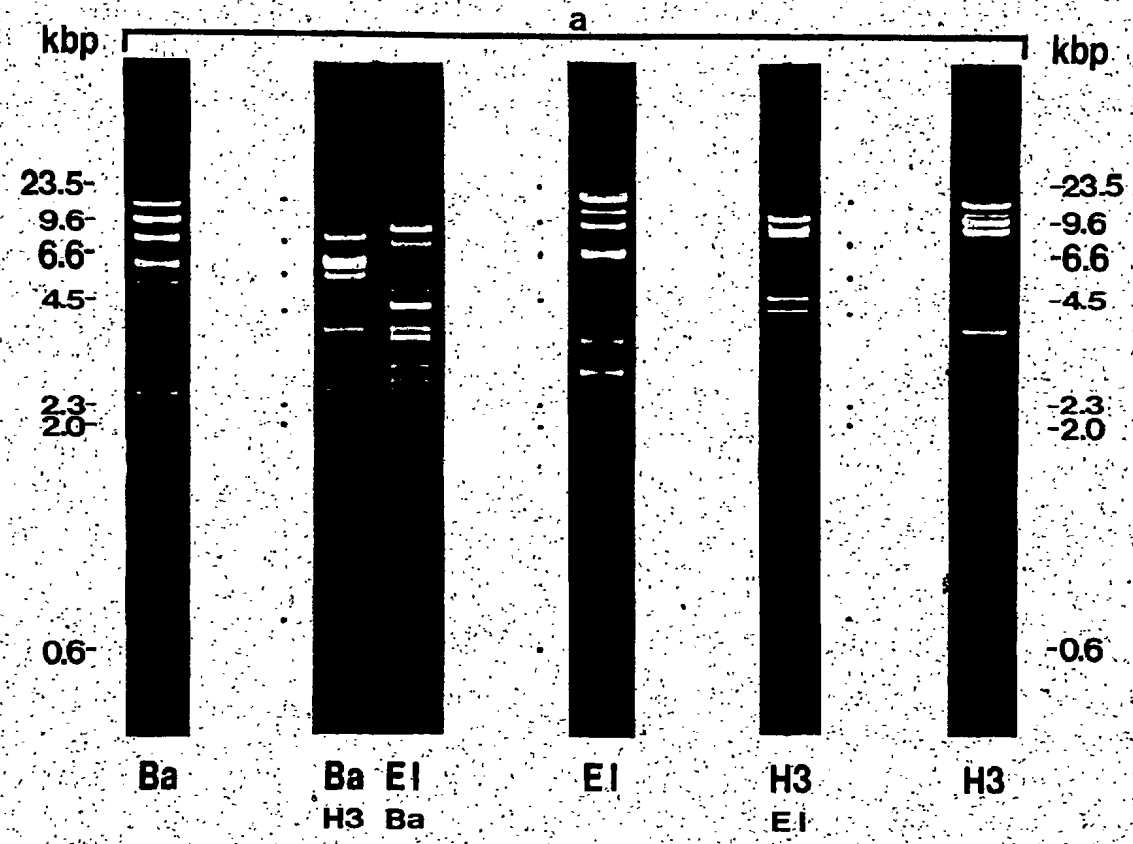
The task of constructing a detailed restriction map of the λ*aroA* genome was approached using an adaptation of the classical double digestion technique. The problems of interpretation inherent to the use of this method were minimised by the selection of restriction enzymes for which the locations of cleavage sites in λpapa DNA are known (96,97).

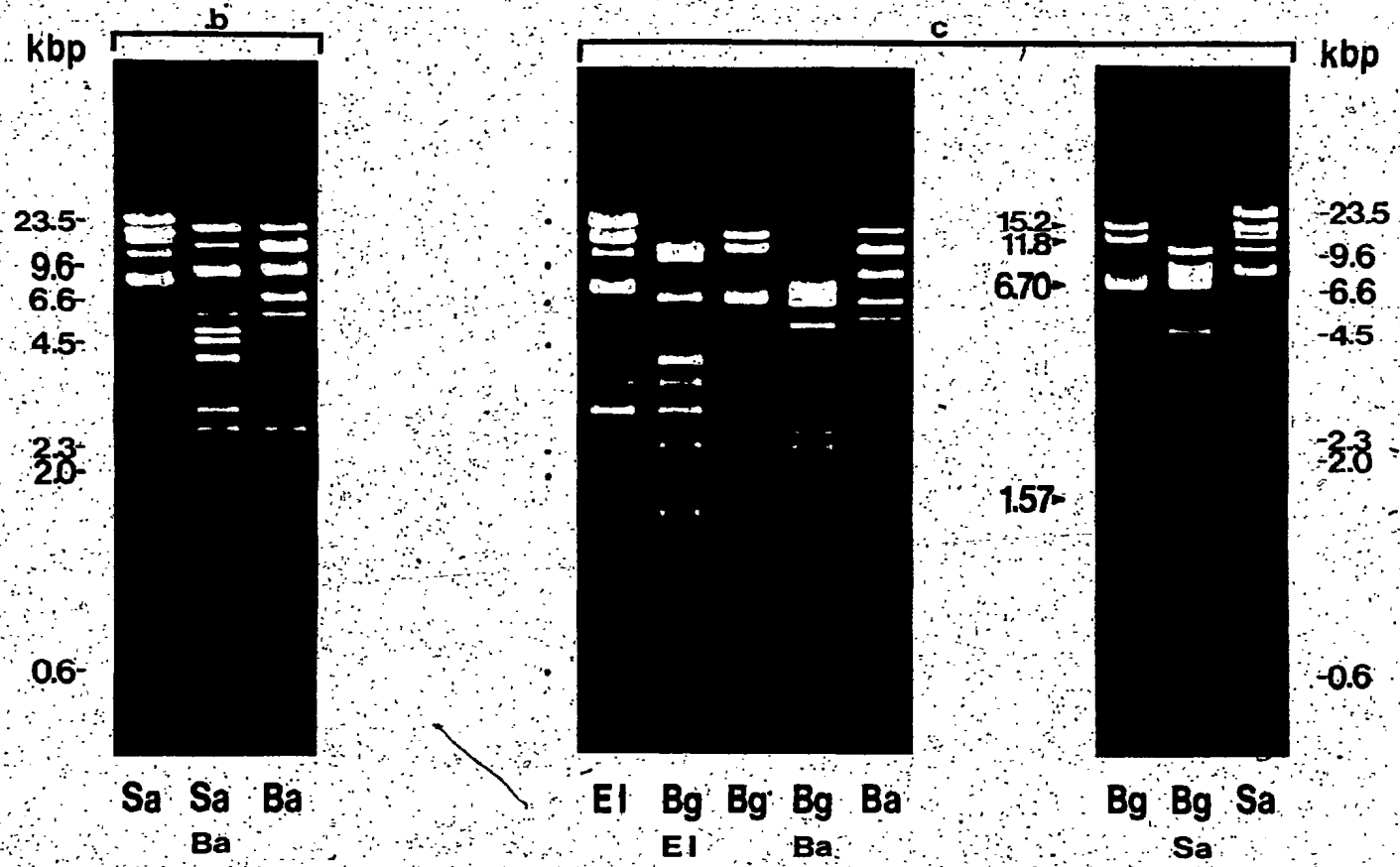
For each restriction enzyme, the patterns of fragments produced in single and appropriate double digestions of λ*aroA* DNA were analysed by agarose gel electrophoresis. The results of these analyses as presented in Figure 4 (a) -(g), were then correlated with a restriction map of the parental λ helper genome deduced from its known relation to the published structure of the λpapa chromosome (5,96), to permit ordering of the fragments found in each digestion.

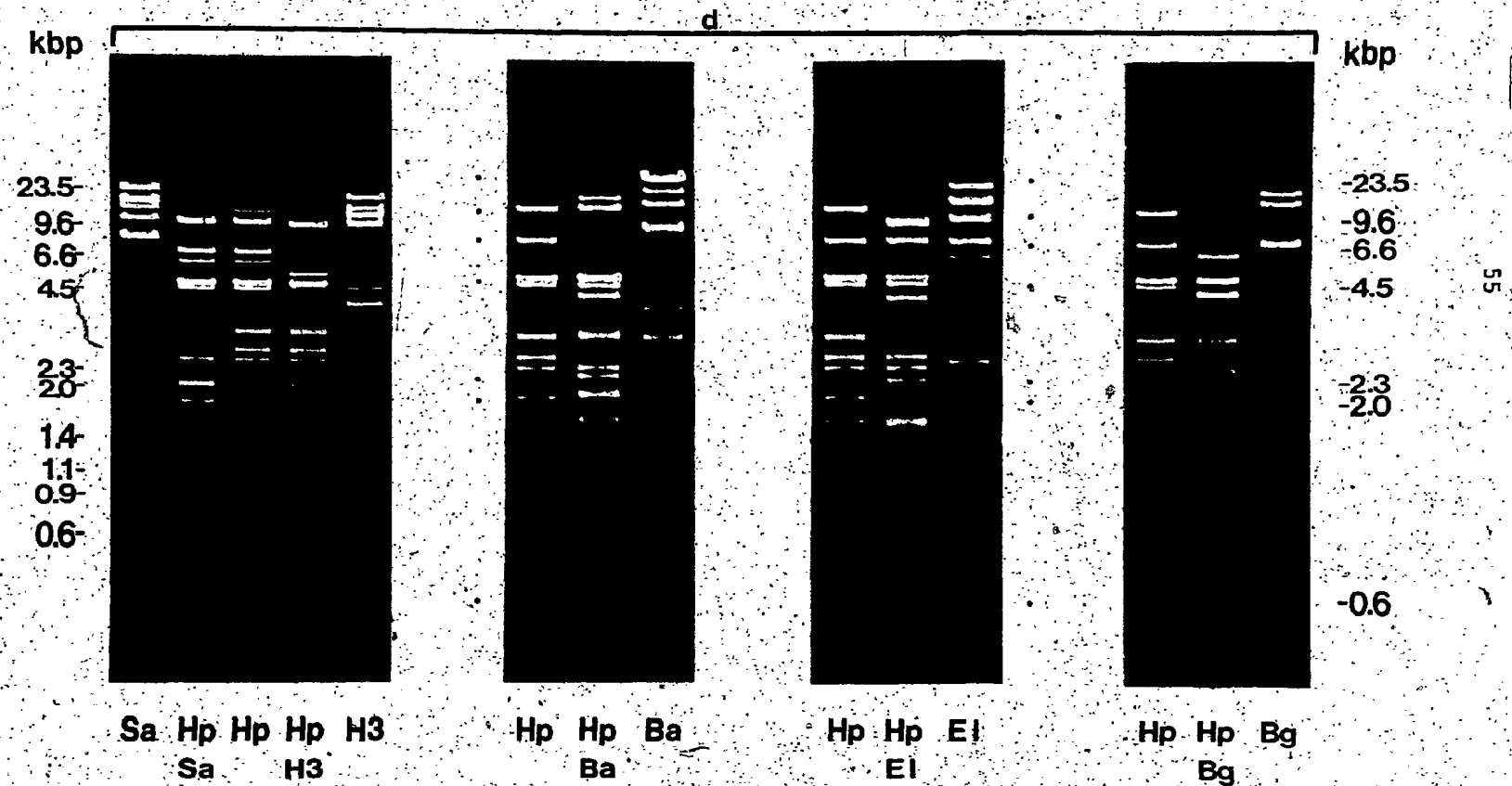
Figure 4. Agarose gel electrophoresis of DNA fragments produced by restriction endonuclease digestion of λ*aroA* DNA.

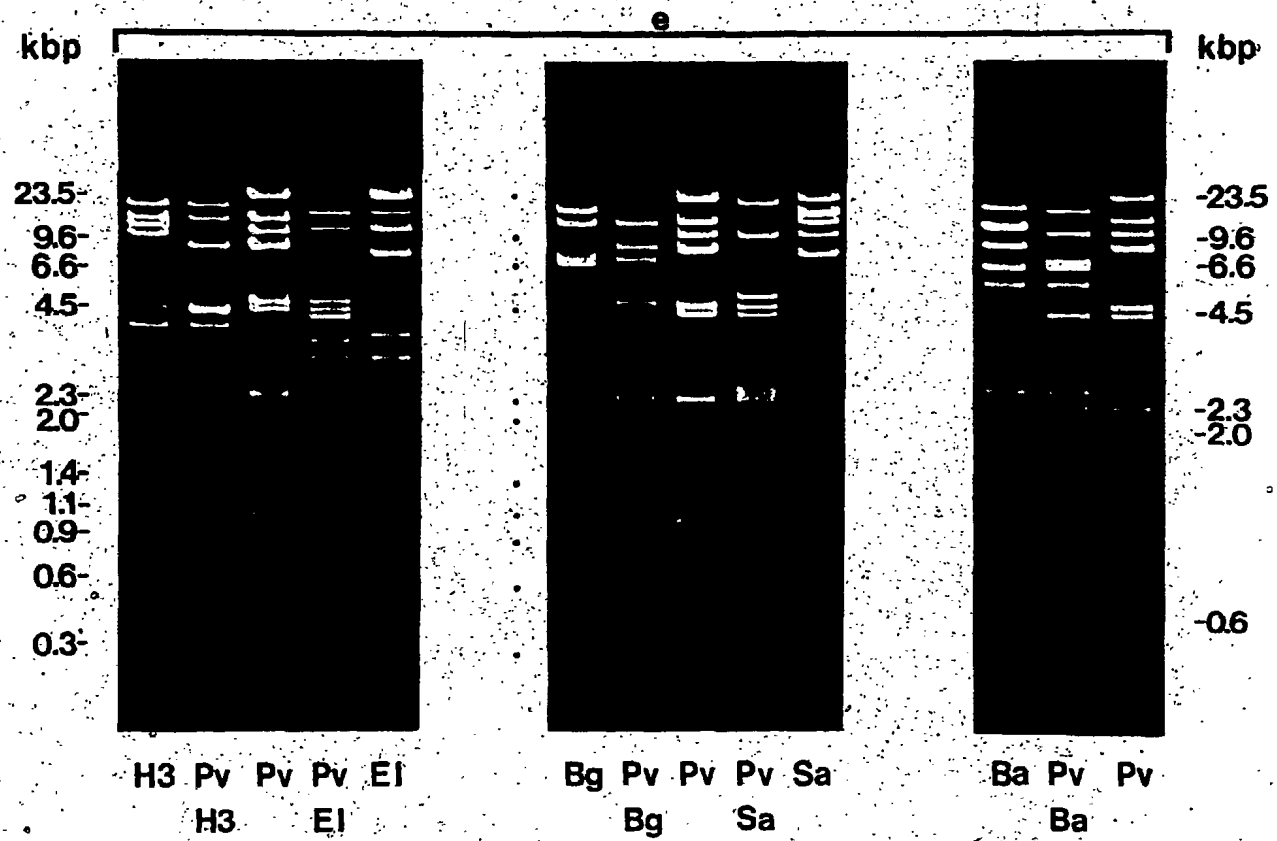
DNA prepared from phage λ*aroA* was digested to completion with single or a combination of restriction enzymes, and the products separated by electrophoresis in 0.8% agarose gels in the presence of ethidium bromide. After running to the desired extent, gels were removed and the fragment patterns obtained photographed using UV illumination (all procedures as described in Methods). Molecular sizes were determined using the following markers; Hin dIII digest of λcI857 (23.51, 9.59, 6.64, 4.45, 2.29, 1.95, 0.59, 0.13 kbp) and Hae III digest of ϕX174 RF (1.35, 1.08, 0.87, 0.60, 0.31, 0.28, 0.27, 0.23, 0.19, 0.12, 0.07 kbp).

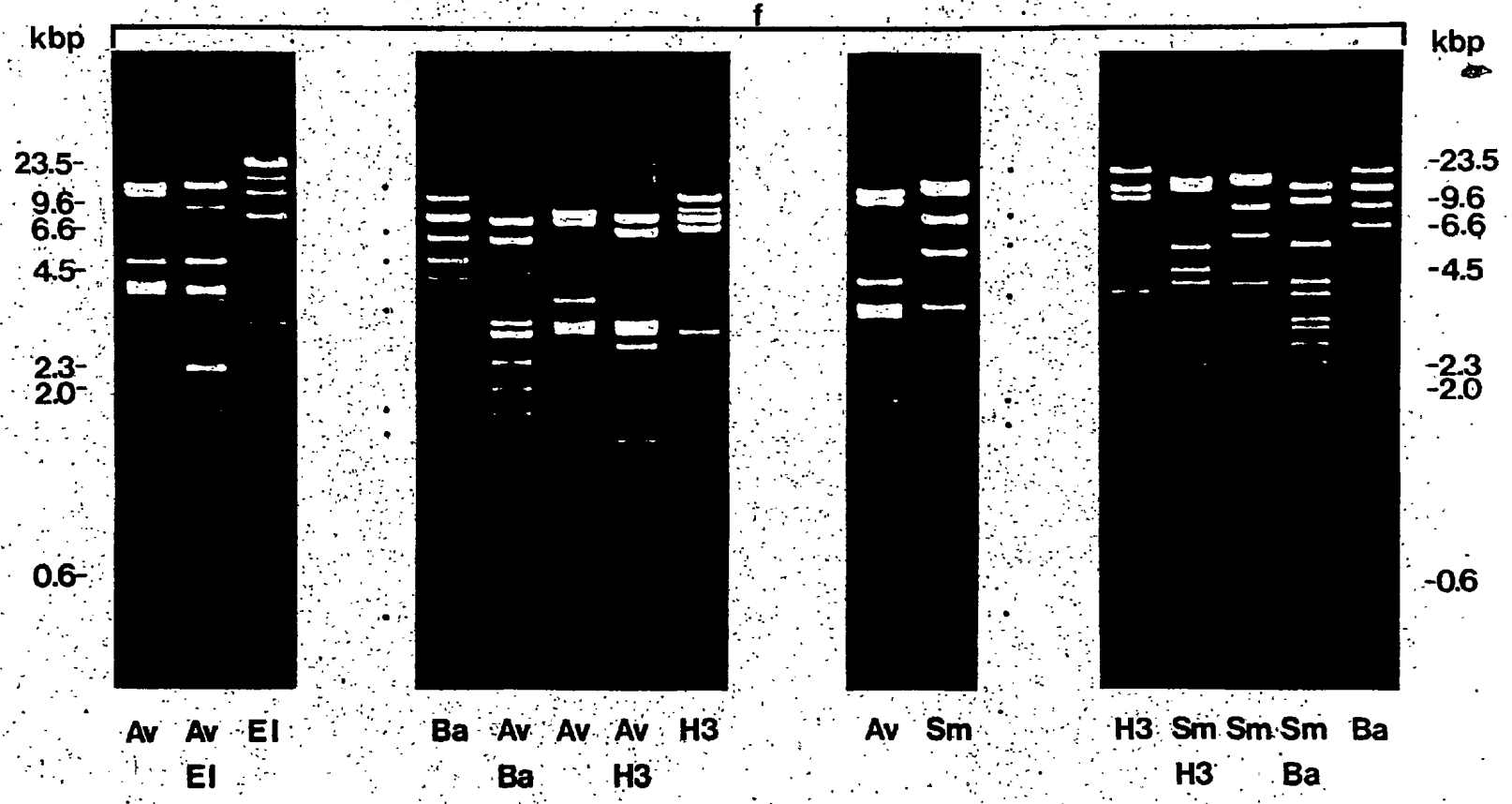
Results pertinent to the mapping of each set of restriction sites are presented grouped according to enzyme; (a) Bam HI, Eco RI and Hin dIII; (b) Sal I; (c) Bgl II; (d) Hpa I; (e) Pvu I; (f) Ava I and Sma I; (g) Sst I.

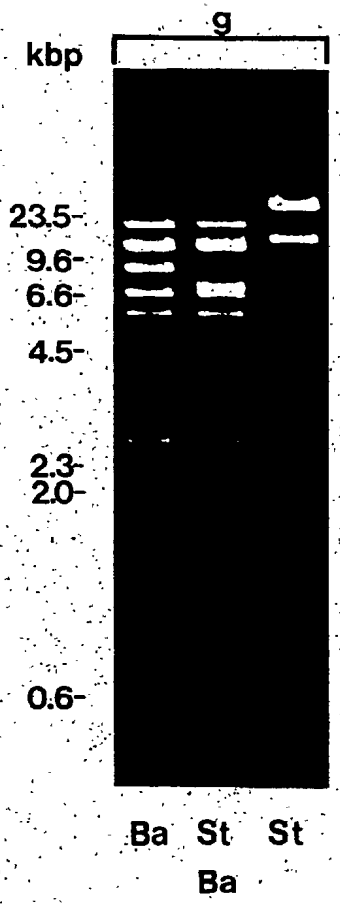

a
kbp
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6
23.5
9.6
6.6
4.5
2.3
2.0
0.6
Ba
Ba
H3
EI
Ba
EI
EI
H3
EI
H3


b
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6
Sa Sa Ba
Ba
c
El Bg Bg Bg Ba
El Ba
15.2
11.8
6.70
1.57
Bg Bg Sa
Sa
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6


d
kbp
23.5
9.6
6.6
4.5
2.3
2.0
1.4
1.1
0.9
0.6
Sa Hp Hp Hp H3
Sa H3
Hp Hp Ba
Ba
Hp Hp El
El
Hp Hp Bg
Bg
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6


e
kbp
23.5
9.6
6.6
4.5
2.3
2.0
1.4
1.1
0.9
0.6
0.3
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6
H3 Pv Pv Pv El
H3 El
Bg Pv Pv Pv Sa
Bg Sa
Ba Pv Pv
Ba


f
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6
kbp
23.5
9.6
6.6
4.5
2.3
2.0
0.6
Av Av EI EI
Ba Av Av Av H3 Ba H3
Av Sm
H3 Sm Sm Sm Ba H3 Ba

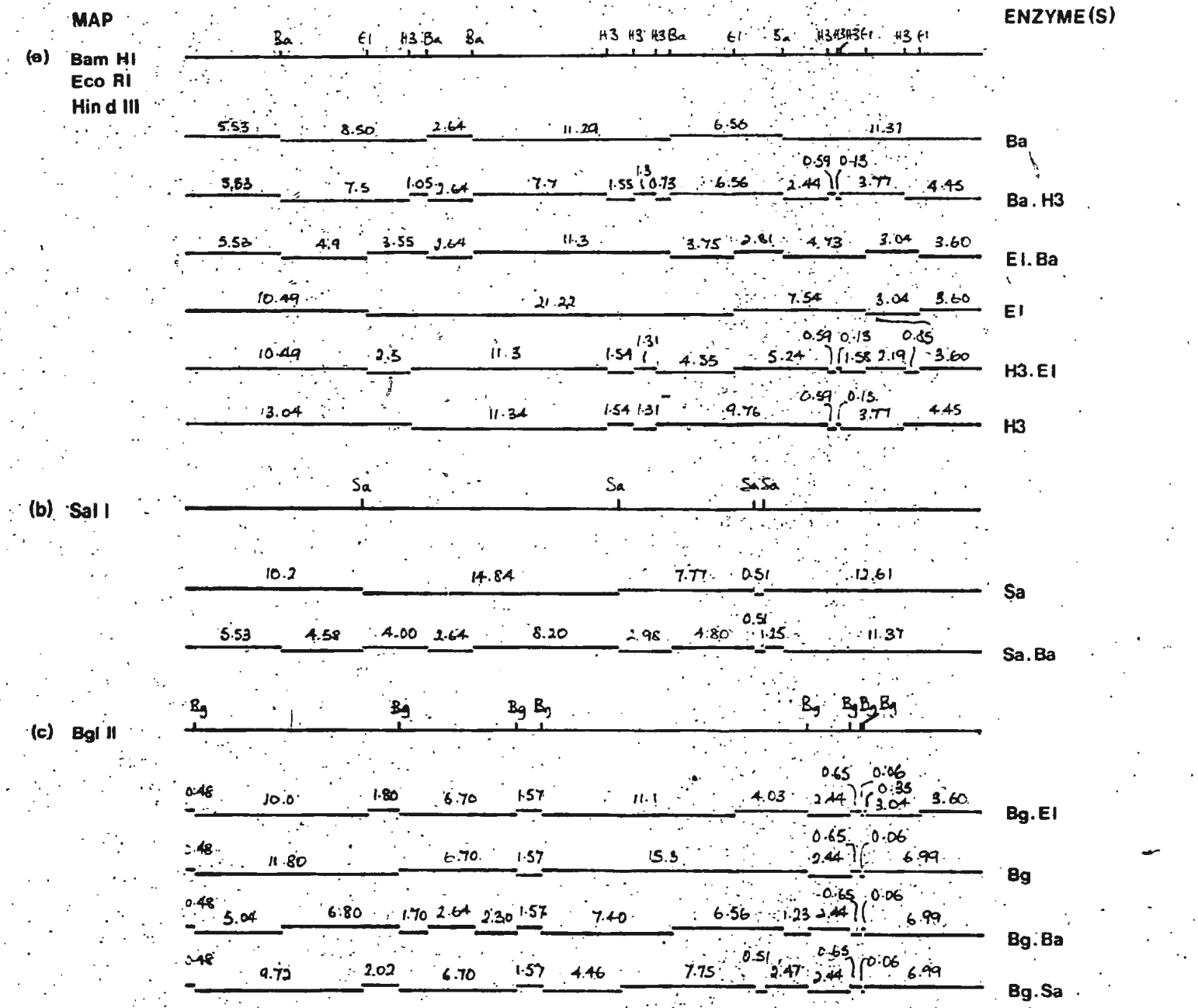

g
kbp
23.5-
9.6-
6.6-
4.5-
2.3-
2.0-
0.6-
Ba
St
St
Ba

Initially, the arrangements of fragments produced by digestion of λaroA DNA with enzymes Bam HI, Eco RI and Hin dIII were determined. The orders shown in Figure 5 (a), were derived from the results of the various double digestions presented in Figure 4 (a), and confirmed at least in their identification of right and left end fragments by the results of an analysis of the effect of thermal melting of λ cohesive ends upon the fragment patterns obtained in each single digestion (data not shown). The orders of restriction fragments produced by digestion of λaroA DNA with enzymes Sal I, Bgl II, Hpa I, Pvu I, Sst I, Ava I and Sma I, as shown in Figure 5 (b)-(g), were then deduced using the preliminary Bam HI-Eco RI-Hin dIII map as a guide to the interpretation of the single and double digest fragment patterns shown in Figure 4 (b)-(g).

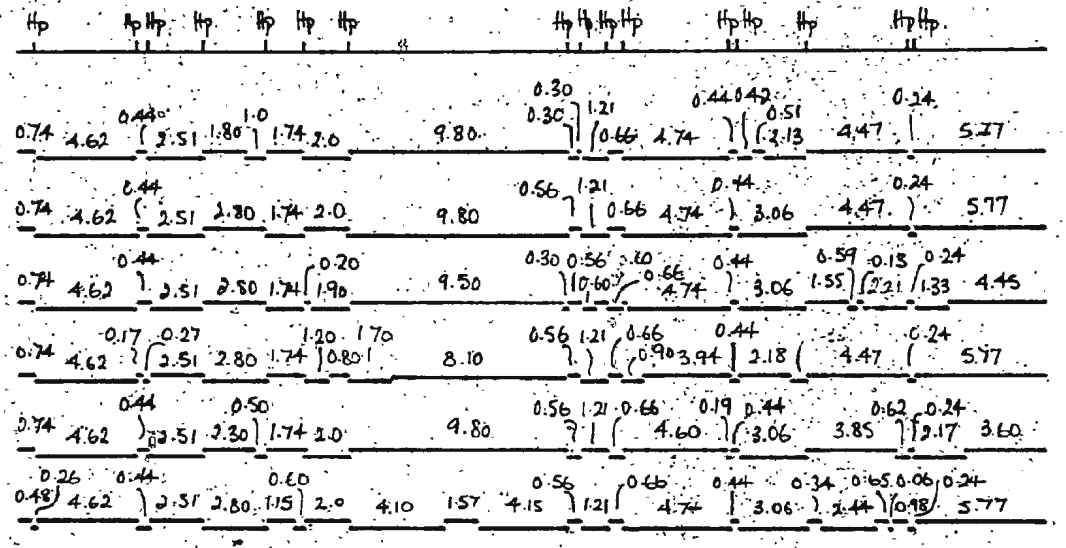
The method by which fragments were ordered using these results may be illustrated in the approach used to construct the Bgl II restriction map of the transducing phage DNA shown in Figure 5 (c). The results of Figure 4 (c) demonstrate that Bgl II digestion of λaroA DNA yields eight detectable fragments of sizes; 15.2, 11.8, 7.0, 6.7, 2.45, 1.57, 0.65 and 0.48 kbp. Of these, four may be identified from the information supplied in published lambda maps as purely λ fragments, and assigned to the far left (0.48) and right arms (2.45, 0.65, 7.0) of the genome. The four remaining fragments, which comprise two of purely

Figure 5. Arrangements of fragments produced by single and double digestion of λ*aroA* DNA with restriction endonucleases.

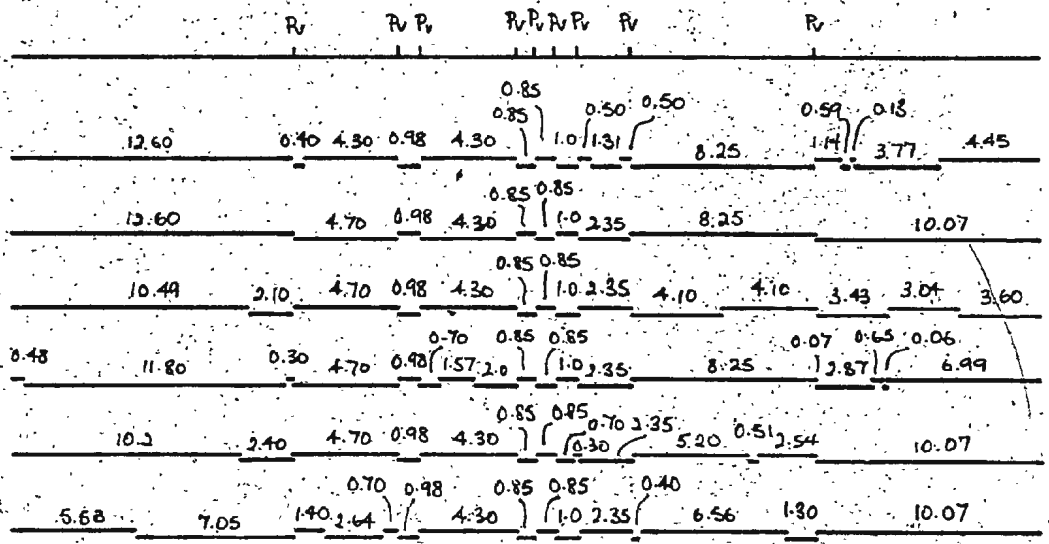
The observations of size and number of restriction fragments produced upon digestion of λ*aroA* DNA with each enzyme and combination of enzymes, as provided by the results of Figure 4 (d)-(g), were collated and the combined information used in the derivation of the fragment orders presented.

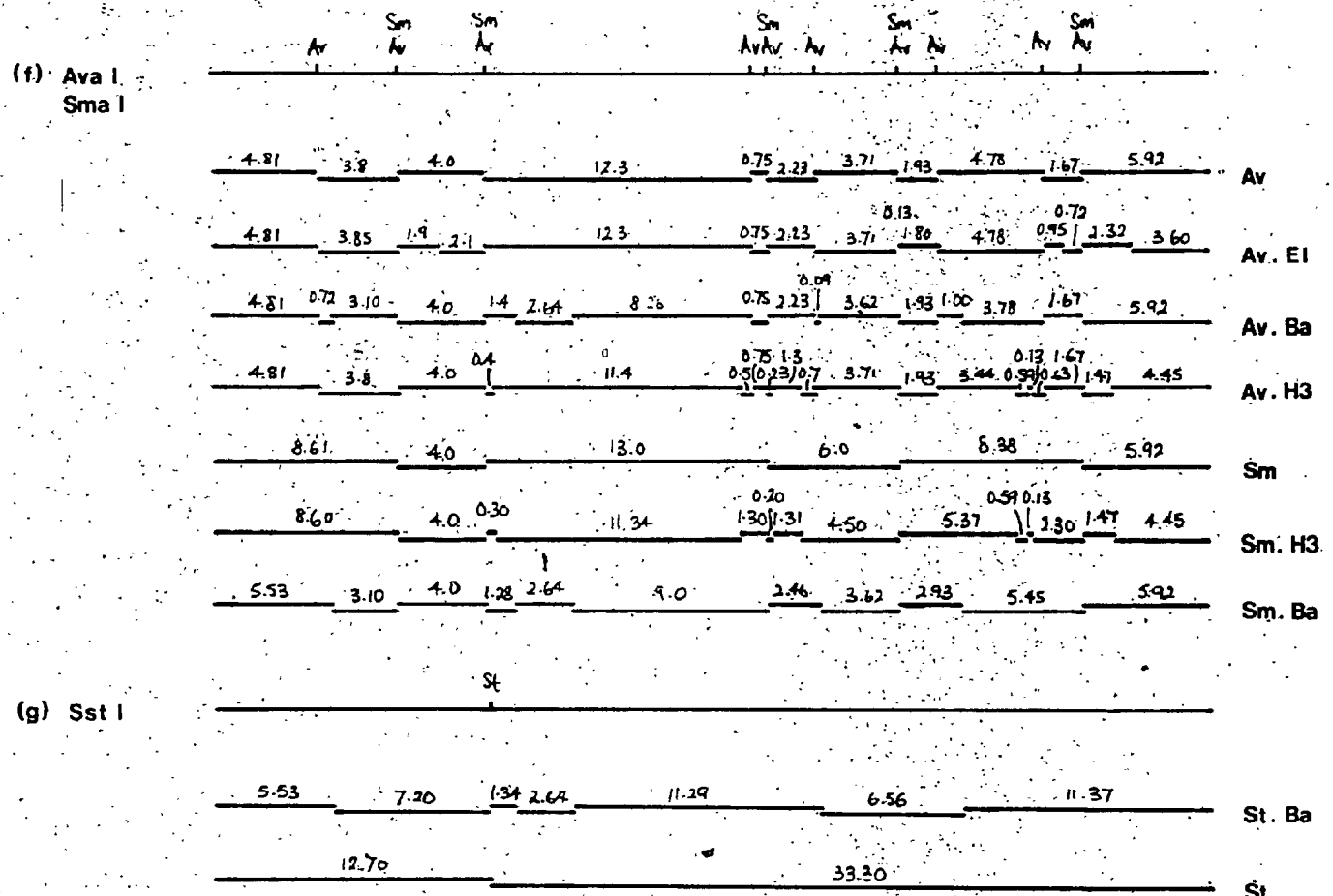

MAP
ENZYME(S)
(a) Bam HI
Eco RI
Hin d III
Ba
Ba . H3
E1 . Ba
E1
H3 . E1
H3
(b) Sal I
Sa
Sa . Ba
(c) Bgl II
Bg . E1
Bg
Bg . Ba
Bg . Sa

(d) Hpa I


Hp
0.74 4.62 0.44 2.51 1.80 1.0 1.74 2.0 9.80 0.30 0.30 1.21 0.66 4.74 0.44 0.42 0.51 2.13 4.47 0.24 5.77
Hp. Sa
0.74 4.62 0.44 2.51 2.80 1.74 2.0 9.80 0.56 1.21 0.66 4.74 0.44 3.06 4.47 0.24 5.77
Hp
0.74 4.62 0.44 2.51 2.80 1.74 0.20 1.90 9.50 0.30 0.56 0.60 0.66 4.74 0.44 3.06 0.59 1.55 0.15 2.21 0.24 1.33 4.45
Hp. H3
0.74 4.62 0.17 0.27 2.51 2.80 1.74 1.20 0.80 1.70 8.10 0.56 1.21 0.66 0.90 3.94 0.44 2.18 4.47 0.24 5.77
Hp. Ba
0.74 4.62 0.44 2.51 2.30 0.50 1.74 2.0 9.80 0.56 1.21 0.66 4.60 0.19 0.44 3.06 3.85 0.62 0.24 2.17 3.60
Hp. E1
0.48 0.26 4.62 0.44 2.51 2.80 0.60 1.15 2.0 4.10 1.57 4.15 0.56 1.21 0.66 4.74 0.44 3.06 0.34 2.44 0.65 0.06 0.98 0.24 5.77
Hp. Bg

(e) Pvu I


Pv
12.60 0.40 4.30 0.98 4.30 0.85 0.85 1.0 0.50 1.31 0.50 8.25 0.59 1.14 0.18 3.77 4.45
Pv. H3
12.60 4.70 0.98 4.30 0.85 0.85 1.0 2.35 8.25 10.07
Pv
10.49 2.10 4.70 0.98 4.30 0.85 0.85 1.0 2.35 4.10 4.10 3.43 3.04 3.60
Pv. E1
0.48 11.80 0.30 4.70 0.98 0.70 1.57 0.85 2.0 0.85 1.0 2.35 8.25 0.07 2.87 0.65 0.06 6.99
Pv. Bg
10.2 2.40 4.70 0.98 4.30 0.85 0.85 0.70 0.30 2.35 5.20 0.51 2.54 10.07
Pv. Sa
5.68 7.05 1.40 0.70 2.64 0.98 4.30 0.85 0.85 1.0 2.35 0.40 6.56 1.30 10.07
Pv. Ba


(f) Ava I
Sma I
Av
Sm
Av
Sm
Av
Av
Sm
Av
Av
Sm
Av
Av
Av
Sm
Av
4.81 3.8 4.0 12.3 0.75 2.23 3.71 1.93 4.78 1.67 5.92
Av
4.81 3.85 1.9 2.1 12.3 0.75 2.23 0.13 3.71 1.80 4.78 0.95 0.72 2.32 3.60
Av. El
4.81 0.72 3.10 4.0 1.4 2.64 8.26 0.75 2.23 0.09 3.62 1.93 1.00 3.78 1.67 5.92
Av. Ba
4.81 3.8 4.0 0.4 11.4 0.75 1.3 0.5 0.23 0.7 3.71 1.93 3.44 0.13 0.58 0.43 1.67 1.47 4.45
Av. H3
8.61 4.0 13.0 6.0 8.38 5.92
Sm
8.60 4.0 0.30 11.34 0.20 1.30 1.31 4.50 5.37 0.59 0.18 2.30 1.47 4.45
Sm. H3
5.53 3.10 4.0 1.28 2.64 9.0 2.46 3.62 2.93 5.45 5.92
Sm. Ba
(g) Sst I
St
5.53 7.20 1.34 2.64 11.29 6.56 11.37
St. Ba
12.70 33.30
St

bacterial and two of part phage and part bacterial sequences, require the interpretation of double digest data for their ordering.

The fragments obtained in Bgl II/Sal I and Bgl II/Eco RI double digestions of the DNA (Figure 4 (c)) both agree in the positions suggested for the two extreme insert containing fragments; 15.2 and 11.8 kbp. In the Bgl II/Eco RI pattern both of these fragments are replaced by the products of their digestion; 15.2 = 11.2 + 4.0 kbp and 11.8 = 10.0 + 1.8 kbp. While in the Bgl II/Sal I pattern they are replaced; 15.2 = 4.46 + 7.75 (Sal I) + 0.51 (Sal I) + 2.47 kbp and 11.8 = 9.72 + 2.02 kbp. The preliminary Eco RI map of λ*aroA* DNA, which gives the precise location of the Eco RI cleavage sites interrupting these Bgl II fragments, pinpoints the position of the 15.2 to the right and the 11.8 fragment to the left end of the insert.

Assuming this assignment, the pattern of fragments produced in a Bgl II/Bam HI double digestion of the DNA (Figure 4 (c)), defines the arrangement of the two remaining purely bacterial Bgl II fragments 6.7 and 1.57, relative to left (11.8) and right (15.2) extremes of the insert. Although the 1.57 kbp fragment is uncut in this double digestion, the 6.7 kbp fragment is replaced by three digestion products; 6.7 = 1.70 + 2.64 (Bam HI) + 2.30 kbp. The identification of the central of these as the Bam HI 2.64 fragment, in light of the preliminary Bam HI map of λ*aroA*

DNA establishes the position of the 6.7 Bgl II fragment on the left side of the 1.57.

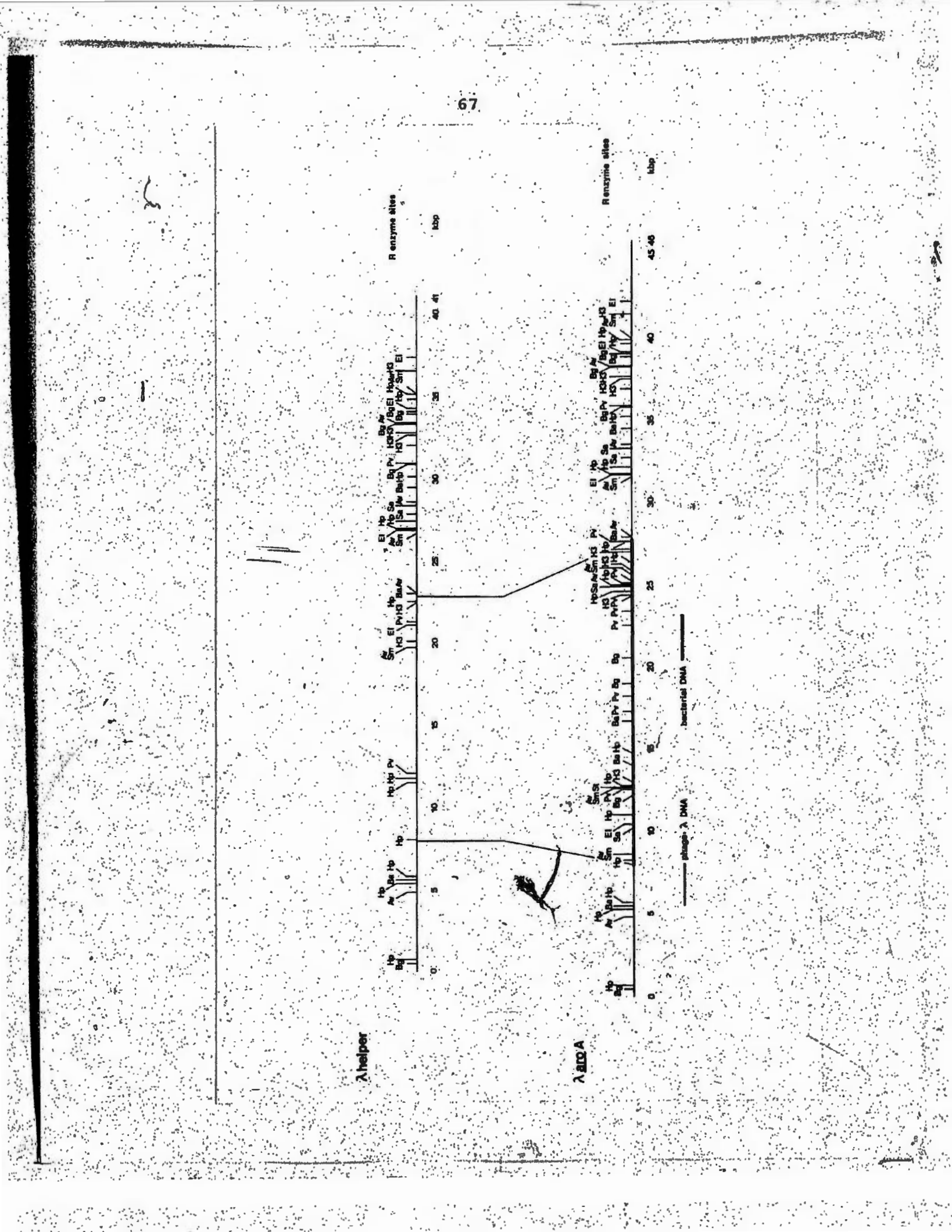
A composite restriction site map of the transducing phage DNA, combining the fragment-order data determined in such a fashion for each digestion was constructed. The resulting detailed description of the λ*aroA* chromosome, when compared to a map of the parental λ helper chromosome derived from published information (5,96), provides a clear idea of the nature and extent of substitution of bacterial for phage sequences in the genome of the transducing phage, as shown in Figure 6. From such a comparison, it is evident that approximately 15 kbp of the lambda sequences immediately left of the att site are lacking in the chromosome of λ*aroA*, replaced in this phage by a segment of the bacterial chromosome measuring some 20 kbp (equivalent to 0.50 *E. coli* chromosome map units), representing 45% of the total λ*aroA* genome.

Detection of DNA fragments in restriction endonuclease digests of *E. coli* chromosomal DNA hybridising to *in vitro* radioactively labeled λ*aroA* DNA

The relationship between the restriction map constructed above of a segment of bacterial DNA contained by a λ*aroA* phage, and the structure of the 20 minute region of the *E. coli* chromosome whence this insert was derived, was explored using the hybridisation technique of Wahl (92).

Figure 6. Restriction enzyme cleavage site map of the λ*aroA* genome.

The orders for restriction fragments obtained in digestions of λaroA DNA with various enzymes as presented in Figure 5 (a)-(g), were combined to produce the composite restriction map shown below. A restriction map of the parental λ helper genome was deduced from its known relation to the published structure of the λpapa chromosome (5, 96).


λhelper
λ arc A
R enzyme sites
kbp
phage λ DNA
bacterial DNA

High molecular weight chromosomal DNA isolated from E. coli C600 (K12) was digested with either Pvu I or Hpa I, and the spectrum of restriction fragments produced electrophoresed in an agarose gel alongside similarly digested DNAs of λ helper and λaroA phages. The separated restricted DNA, as shown in Figure 7 (a), was then transferred by Southern blot to nitrocellulose, and hybridised to λaroA DNA previously labeled with dCTP (γ-^{32}P) to high specific activity by nick translation.

The results of this hybridisation, presented in Figure 7 (b), permit a comparison of the overall arrangements of sequences in the λaroA bacterial insert and corresponding E. coli chromosomal segment. Among the Pvu I and Hpa I fragments present in the digests of chromosomal DNA, several were found to hybridise efficiently with the labeled λaroA DNA.

In the Pvu I chromosomal digestion, nine fragments hybridised with this probe, including seven; 4.7, 4.3, 2.35, 1.0, 0.98, 0.85 and 0.85 kbp, which comigrate with products obtained in the like digest of transducing phage DNA. The Pvu I map of λaroA DNA presented in Figure 8 shows that these seven fragments combined represent the entire internal portion of the bacterial insert. The remaining two hybridising fragments of 6.0 and 13.0 kbp replace the part bacterial part phage Pvu I fragments of λaroA

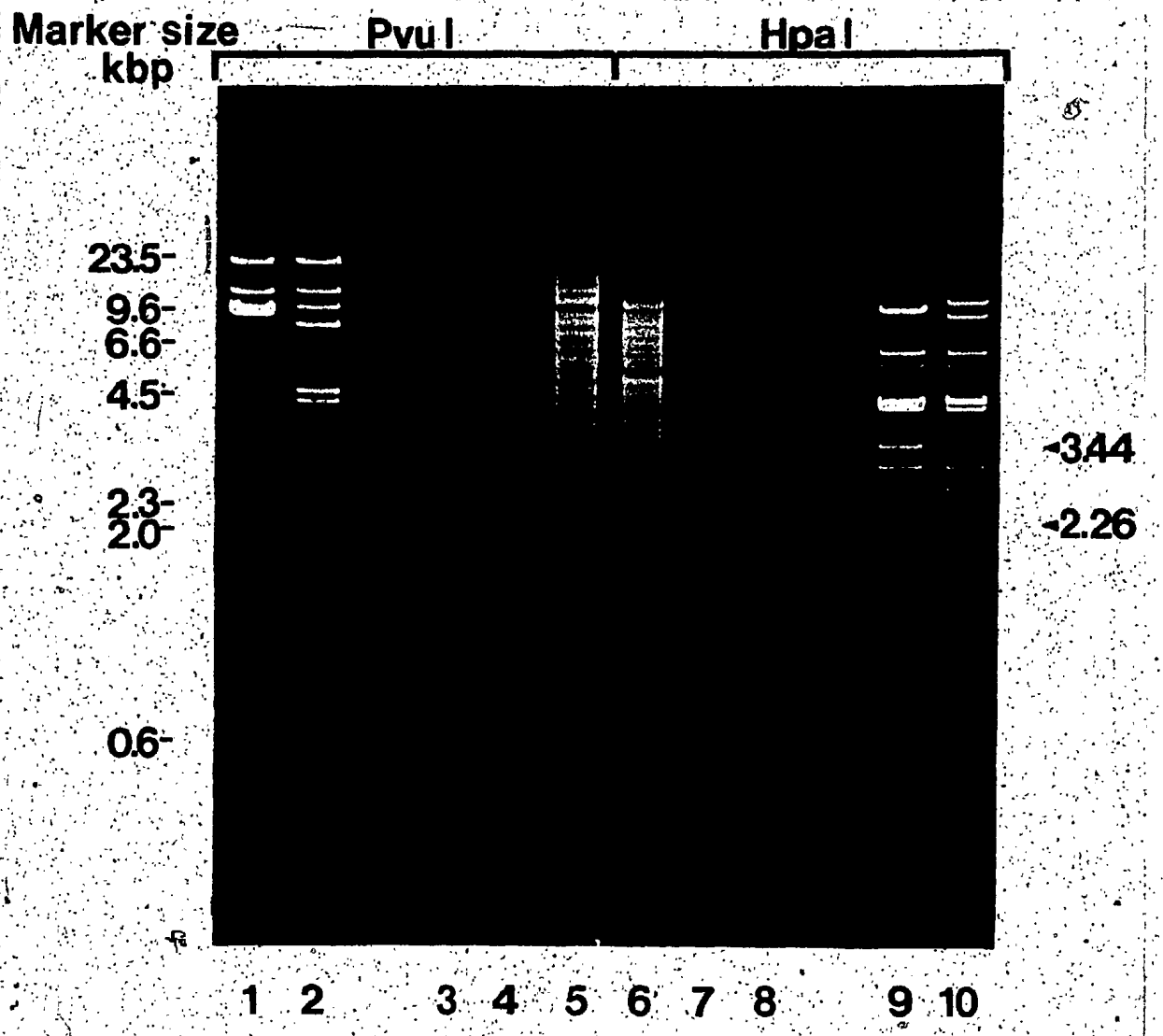
Figure 7. Hybridisation of E. coli C600 chromosomal DNA digested with restriction endonucleases to in vitro radioactively labeled λaroA DNA.

E. coli chromosomal DNA was prepared using conventional methods, digested to completion with either Pvu I or Hpa I and the restricted DNA separated by electrophoresis in a 0.8% agarose gel alongside markers representing the products of like digestions of λ helper and λaroA DNAs.

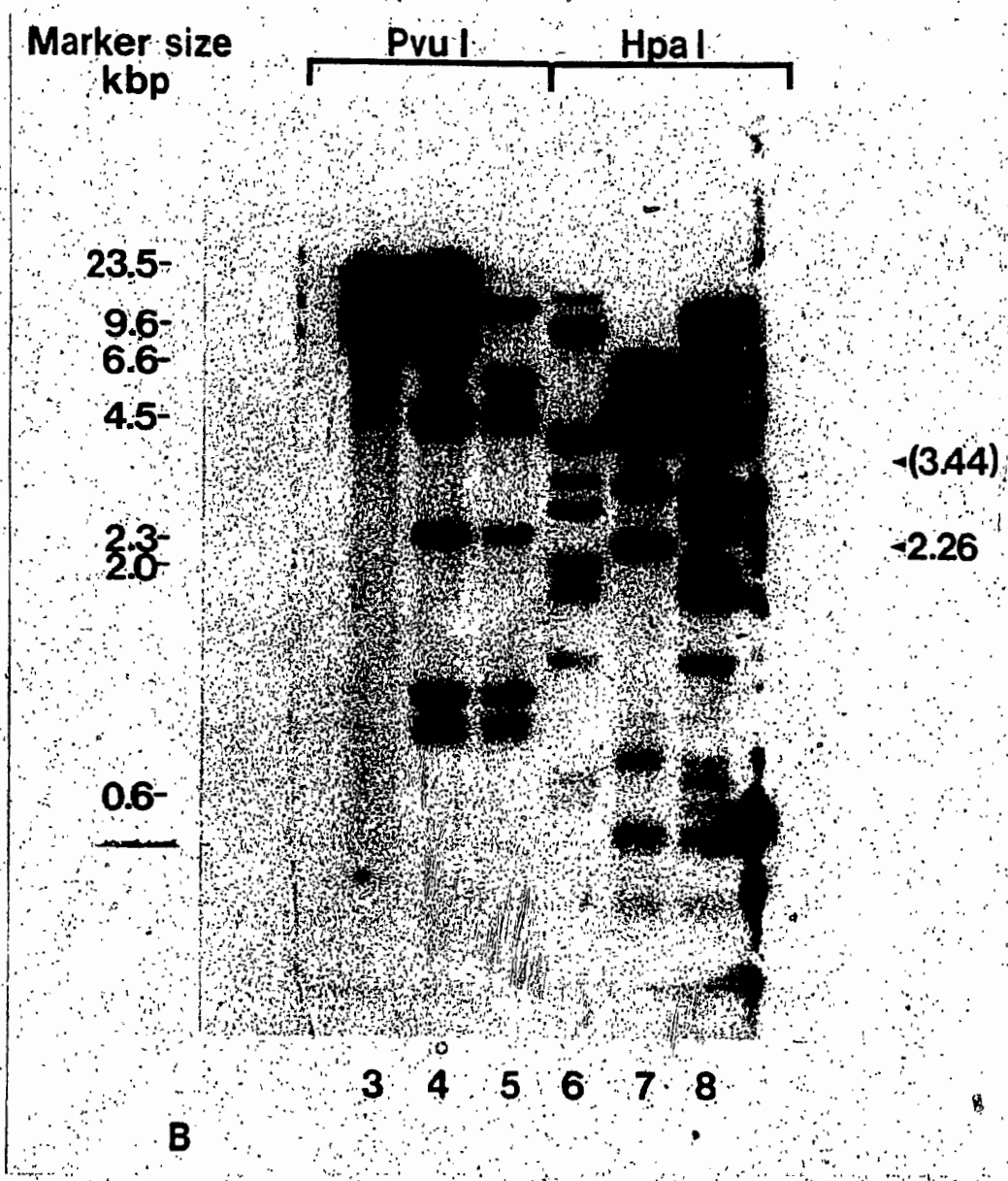
The ethidium bromide staining pattern of fragments is shown in part (a); Pvu I digestions: lanes 1 & 3 λ helper DNA (0.5 μg & 25 ng); lanes 2 & 4 λaroA DNA (0.5 μg & 25 ng); lane 5 E. coli C600 DNA (2.5 μg), and Hpa I digestions: lane 6 E. coli C600 DNA (2.5 μg); lanes 7 & 9 λ helper DNA (25 ng & 0.5 μg); lanes 8 & 10 λaroA DNA (25 ng & 0.5 μg).

The restriced DNA was cleaved to small fragments, denatured and blotted from the gel onto a sheet of nitrocellulose. After baking and prehybridisation, the paper was treated with nick-translated dCTP (γ-^{32}P) labeled probe λaroA DNA for a period, then washed extensively at room temperature then 42°C in high then low salt.

The hybridisation pattern of fragments obtained upon autoradiographic exposure of the blot is shown in part (b); Pvu I digestions: lane 3 λ helper DNA (25 ng); lane 4 λaroA DNA (25 ng); lane 5 E. coli C600 DNA (2.5 μg), Hpa I digestions: lane 6 E. coli C600 DNA (2.5 μg); lane 7 λ helper DNA (25 ng); lane 8 λaroA DNA (25 ng). (Film exposure 36 hr). (All procedures as described in Methods). Molecular sizes were determined using the following markers; Hin dIII digest of λcI857 (23.51, 9.59, 6.64, 4.45, 2.29, 1.95, 0.59, 0.13 kbp).


Marker size
kbp
Pvu I
Hpa I
23.5-
9.6-
6.6-
4.5-
2.3-
2.0-
0.6-
3.44
2.26
1 2 3 4 5 6 7 8 9 10

A


Marker size
kbp
Pvu I
Hpa I
23.5-
9.6-
6.6-
4.5-
2.3-
2.0-
0.6-
(3.44)
2.26
3 4 5 6 7 8
B

containing left (12.6) and right (8.25) ends of the insert.

Similarly, in the Hpa I chromosomal digestion, nine fragments hybridised with the probe, seven of which; 9.8, 2.80, 2.00, 1.74, 1.21, 0.66 and 0.56 kbp comigrate with λ*aroA* Hpa I digestion products. The Hpa I map of λ*aroA* DNA shown in Figure 8 shows that these seven fragments together represent the internal section of the bacterial insert carried by this transducing phage, confirming the Pvu I results. The two additional hybridising fragments of 4.01 and 3.20 kbp once again replace λ*aroA* Hpa I fragments containing left (2.51) and right (4.74) insert ends. An 11.8 kbp Hpa I fragment which appears in both ethidium bromide stain and hybridisation patterns of λ*aroA* digestion products probably represents the combination of 9.8 + 2.00 kbp insert fragments, which are separated by a Hpa I site believed partially resistant to cleavage.

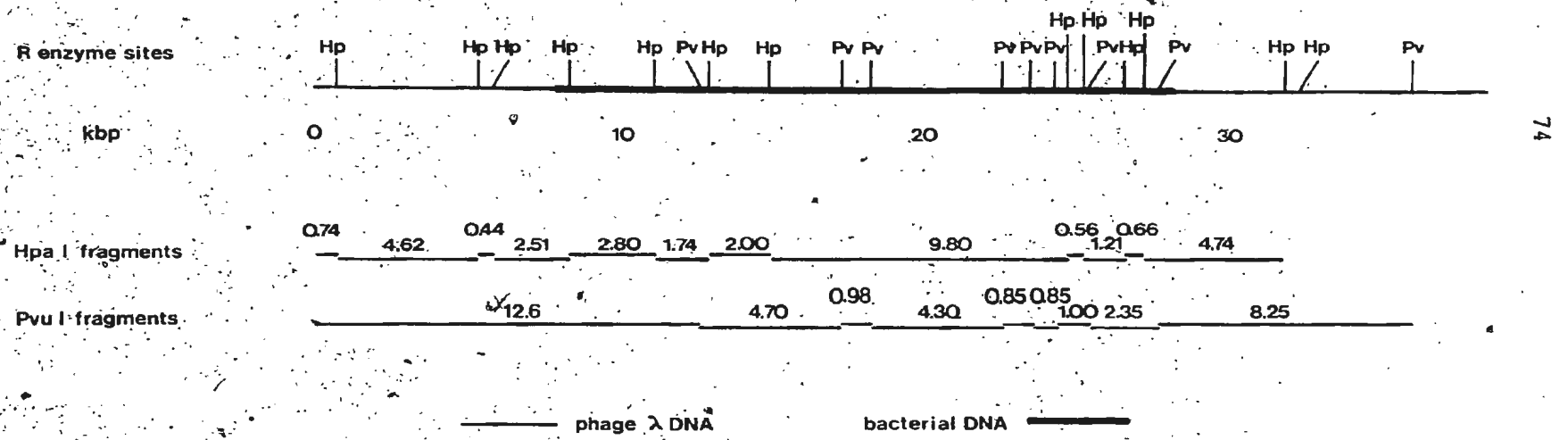
The combination of the hybridisation results, when viewed as in Figure 8 in terms of the established Pvu I and Hpa I restriction maps of λ*aroA* DNA, strongly supports the proposition that the overall arrangement of DNA sequences described for the λ*aroA* bacterial insert truly reflects that found in the region of the *E. coli* chromosome from which it was derived.

The data of Figures 7 (a) and (b) also provide for further comparison of the structures of λ helper and λ*aroA* genomes. The majority of the fragments produced by Pvu I

Figure 8. Hpa I and Pvu I restriction enzyme cleavage site maps of the left of the λ*aroA* genome.

The order of Hpa I and Pvu I restriction fragments of λ*aroA* DNA described previously (see Figure 5 (d) & (e)), are reproduced below in relation specifically to the structure of the left arm of the λ*aroA* genome.

λ aro A


R enzyme sites
Hp
Hp Hp
Hp
Hp
Pv Hp
Hp
Pv Pv
Pv
Pv Pv Pv
Pv Hp
Hp Hp Hp
Pv
Hp Hp
Pv
kbp
0
10
20
30
Hpa I fragments
0.74
4.62
0.44
2.51
2.80
1.74
2.00
9.80
0.56
0.66
1.21
4.74
Pvu I fragments
12.6
4.70
0.98
4.30
0.85
0.85
1.00
2.35
8.25
phage λ DNA
bacterial DNA

and Hpa I digestion of λ helper DNA hybridise with the radioactively labeled λ*aroA* DNA, as would be expected from the extent of sequences common to both phages (Figure 6). However, the pattern of hybridisation to digested λ helper DNA shown in Figure 7 (b) differs in one respect from that anticipated. The digestion of λ helper DNA with Hpa I (Figure 7 (a)), yields fourteen detectable fragments, arranged in the genome of the phage in the order (right to left); 6.51 (the combination of left and right end fragments), 0.74, 4.62, 4.47, 3.44, 2.26, 0.25, 10.7, 4.52, 0.44, 3.06, 4.47, 0.24, 5.77 kbp. The substitution of bacterial for phage sequences in the derived λ*aroA* transducing phage genome removes a segment of lambda DNA, which from the published Hpa I map of the λpapa chromosome (98,96) should include part of 3.44 and all of fragments 2.26, 0.25 and 10.7. As a consequence, it might be expected that fragments 2.26, 0.25 and 10.7 though present in the pattern of products of Hpa I digestion of λ helper DNA shown in Figure 7 (a), would not hybridise with radioactively labeled λ*aroA* DNA, while fragment 3.44 (which contains some sequences common to the transducing phage genome) would.

The results of Figure 7 (b) however contradict this expectation, to the extent that the 2.26 kbp fragment of λ helper does hybridise to the labeled probe, while the 3.44 kbp fragment does not. This discrepancy may indicate that some revision of the published Hpa I restriction site map of λpapa might be necessary to accomodate a reversal

in the order of these two fragments; G (3.44) and I (2.26) (98).

DISCUSSION

The transducing phage λ*aroA* contains the gene for ribosomal protein S1

The transducing phage λ*aroA* has been shown to encode a product identified as r-protein S1 on the basis of essentially two criteria. First, the demonstrated comigration of a protein synthesised in λ*aro*-infected cells with pure S1 by SDS and later O'Farrell two dimensional polyacrylamide gel electrophoresis, which establishes the identity of this material according to M_r and isoelectric point. Second, the observed specific precipitation from extracts of λ*aroA*-infected cells reacted with anti S1 serum of a protein, shown by SDS polyacrylamide gel electrophoresis to be this comigrating product, which demonstrates the identity of this comigrating species from the standpoint of immunological reactivity.

The identification of r-protein S1 among the products encoded by a λ*serC* transducing phage has recently been reported (11). In this case, identification rested upon demonstrated comigration of a species synthesised in cells infected with phage λ*serC* with an r-protein occupying a position characteristic of S1 in the two dimensional polyacrylamide gel electrophoretic system of Li & Subramanian

(99). The observed ability of λserC to transduce an aroA⁻ strain to aro⁺ prototrophy (11), would indicate that this transducing phage contains bacterial DNA common to the λaroA phage studied here, indirectly confirming the identification of S1 among products encoded by the latter.

Although it is unclear from the published results whether the promoter governing expression of the gene for r-protein S1 (rpsA) carried by λserC is a phage or bacterial activity (11), this study has established that the promoter directing synthesis of S1 in λaroA-infected cells is of a bacterial origin. The basis for this conclusion lies in observed immunity of λaroA-encoded synthesis of S1 from cI repression, in a homoimmune lysogen infected with this phage, in which phage-promoted expression is virtually eliminated. Such a criterion has been applied frequently in the past in the identification of promoter activities governing expression of r-protein genes carried by transducing and recombinant phages (16,39,42). The identification of this bacterial activity as the normal chromosomal promoter of rpsA is supported by the results of an analysis comparing arrangements of bacterial sequences in the insert of λaroA and the chromosomal context from which the insert was derived.

The relative intensity of the bacterial bands observed in electrophoretic analyses of material synthesised in the λaroA-infected lysogen, which would appear from the simil-

arities between band patterns obtained with alternative (^{14}C) lysine and (^{35}S) methionine labels not to reflect simple differences in the relative proportions of the labeling amino acids in these proteins, suggests that this putative rpsA promoter is of a comparatively high efficiency.

Other bacterial genes contained by the transducing phage λaroA

The observed synthesis in λaroA-infected cells of six proteins unique to the transducing phage infection has been interpreted as suggesting that this phage encodes several other bacterial proteins in addition to that identified as r-protein S1. Although certain identification of these species based upon the present results is not possible, some comment upon their likely or unlikely identities is appropriate.

The two bacterial markers, aroA and rpsA, known to be carried by phage λaroA from the procedure used in the selection of this transducing phage (5; R. Weisberg, personal communication) and the results of this analysis respectively, both map at positions close to 20 minutes on the E. coli linkage map (100). This knowledge defines the portion of the chromosome from which the insert of bacterial DNA contained by this phage was derived. The possibil-

ity that other genes located in this chromosomal region might also be carried by this transducing phage depends essentially upon two considerations. First, the position of each gene in relation to the site of integration of the λ parent of this phage and location of the bacterial marker, aroA, employed in the selection of the phage from a generalised transducing lysate. Second, the distance between each gene and the site of λ integration relative to the length of the bacterial insert contained by the λaroA genome.

Several lines of evidence indicate that the secondary attachment site preferred for integration of λ into the chromosome at the 20 minute region is situated on the right (clockwise) and close to the rpsA locus. This assignment would explain the observation that all three λ transducing phages isolated using serC as the selected marker, were found also to contain genes aroA and rpsA (10). In addition it would suggest the possibility, given an appropriate choice of selective marker, of obtaining transducing phages containing loci to the right of rpsA only. This prediction, with the recent characterisation of λompF phages shown to contain aspC and asnS but not aroA (101) and the earlier reported isolation of λasnS phages shown not to encode S1 (102), appears to have been fulfilled.

If this assignment of the site of λ integration is correct, the possibility that chromosomal loci right of

rpsA might be contained in the insert carried by λ*aroA* would appear very unlikely. Instead, it would be expected that bacterial proteins encoded by this phage would represent products of genes, mapped and unmapped, located to the left (anticlockwise) of *rpsA* only.

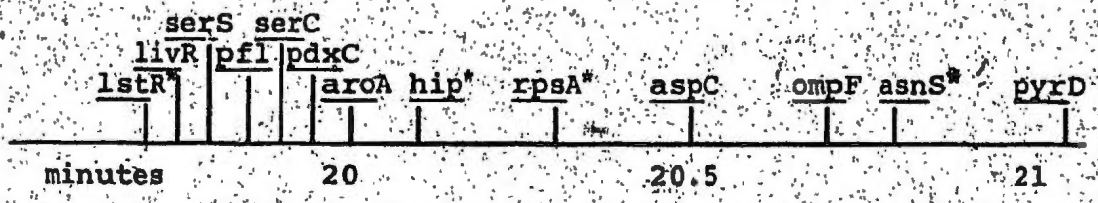
The length of the segment of bacterial DNA carried by λ*aroA* has been estimated from the results of detailed restriction mapping of the phage DNA to be 20 kbp, or 0.50 minutes of the *E. coli* chromosome. This measurement precisely defines the distance from the site of λ integration beyond which loci are unlikely to have been included in the transducing phage genome. Combining this information with the position of λ integration assumed above would point to the probable source of bacterial genes contained by λ*aroA* as deriving from the region of the chromosome between 19.5 and 20.5 minutes of the linkage map.

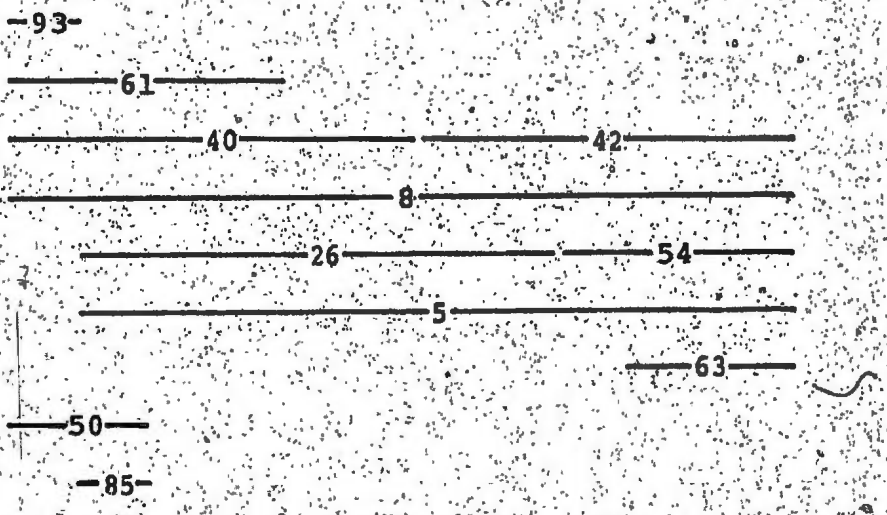
Genes mapping at the 20 minute region of the *E. coli* chromosome

The arrangement of genetic loci mapping to the 20 minute region of the *E. coli* chromosome, which is known in some detail (Figure 9; 100), would suggest a number of possible identities for the bacterial proteins encoded by phage λ*aroA*. The identification of proteins corresponding to these genes among λ*aroA*-encoded products is however somewhat hampered by the lack of published information, specif-

Figure 9. Arrangement of genetic loci at the 20 minute region of the *E. coli* chromosome.

The arrangement of loci presented, in which asterisks are used to identify mapped loci whose position relative to adjacent markers is not precisely known, is adapted from the updated *E. coli* K12 linkage map (100). The cotransduction frequencies shown are derived from original sources (21,103-109).


serS serC
livR pfl pdxC
lstR* aroA hip* rpsA* aspC ompF asnS* pyrD
minutes
20
20.5
21


93
61
40
42
8
26
54
5
63
50
85

ically of M_r and behaviour in O'Farrell two dimensional polyacrylamide gel electrophoresis, which would allow a direct comparison of their properties.

The results of complementation analyses of the loci aroA and hip previously performed (R. Weisberg, personal communication), would suggest that products corresponding to aroA (3-enolpyruvylshikimate-5-phosphate synthetase) and hip (host integration protein), might be among those encoded by this phage. The possibility that these loci though present might be incomplete and unexpressed in the transducing phage is however not excluded by such evidence.

Of the proteins coded for by the 20 minute region for which either M_r or migration in O'Farrell electrophoresis is documented (95,110), two exhibit properties similar to those described for λaroA-encoded products (Figures 1 & 3). These proteins, whose published characteristics resemble those determined for proteins of M_r = 45,500 and 39,000 encoded by the phage, correspond to loci serS (seryl tRNA synthetase, EC 6.1.1.11) and aspC (aspartate aminotransferase, EC 2.6.1.1) respectively. It must be noted however, that the assignment of the identity of aspC protein to a product encoded by λaroA would contradict evidence cited earlier as a basis for defining the site of integration used in the creation of this phage.

Unmapped ribosomal protein genes

In view of the observed clustered distribution of ribosomal genes at several chromosomal loci (28,29,34,37; reviewed in 111), it would appear worthwhile to consider the possibility that r-protein genes as yet unmapped, might be closely linked to rpsA. At present only four of the fifty-two genes for r-proteins in E. coli remain to be located on the chromosome, those corresponding to proteins S9, L13, L20 and L34 (20,38). The possible linkage of one of these loci with rpsA, that encoding S9, has recently been suggested on the grounds of the protein's importance for assembly of S1 into reconstituted ribosome particles (112,81).

One approach that may be adopted toward answering such a question of linkage is the analysis of proteins encoded by phage λaroA for species resembling the products of these unmapped genes. A comparison of the range of M_r exhibited by phage-coded bacterial proteins determined in Figure 1 (29,500-80,000), with that reported for these r-proteins estimated using similar SDS polyacrylamide gel electrophoretic techniques (113; S9, 16,200; L13, 17,800; L20, 17,200; L34, 9,600) would seem to rule out the possibility that λaroA might contain the corresponding genes. Although protein L20 lacks methionine (114), the similarity between phage product patterns obtained with alternati-

ve (^{35}S) methionine and (^{14}C) lysine labels would suggest that it also is unlikely to be encoded by phage λ*aroA*. The results of a similar analysis of proteins encoded by λ*serC* employing the two dimensional polyacrylamide gel electrophoretic system of Kaltschmidt & Wittmann (115), may be interpreted as confirmation of this conclusion (11).

This evidence does not however completely rule out the possibility that one or several of these r-protein genes might be located close to or even within the same transcriptional unit as *rpsA*. Such a conclusion would require a more precise knowledge of the position within the bacterial insert contained by this phage of the *rpsA* gene. If the preferred site of λ integration into the 20 minute chromosomal region is very close to and on the right of *rpsA*, as has been suggested above, it would remain a possibility that loci situated rightward of this site might include such unmapped r-protein genes. An analysis of the proteins encoded by λ*ompF* phages might possibly be illuminating in this regard.

Physical structure of the λ*aroA* genome

A restriction map of the λ*aroA* genome has been constructed from the results of single and double digestions of the phage DNA performed using a variety of restriction

endonucleases. The degree of internal consistency exhibited by this map, as shown in Figure 6, would suggest that it is an accurate representation of the structure of the phage genome. Comparison of the arrangement of bacterial sequences contained within the phage genome with that of the chromosomal region from which it was derived, has indicated that the structure determined for the phage insert truly reflects that prevailing in the *E. coli* chromosome.

A calculation of the combined molecular weights of bacterial proteins encoded by λ*aroA* (M_r = 327,000), suggests a minimum size for the insert contained by this phage of 9 kbp, or 45% of the size indicated by the restriction map of the λ*aroA* genome. This discrepancy might indicate the existence of extensive non-coding regions within the insert, or alternatively reflect a content of bacterial genes expressed at low and undetectable levels under the conditions of analysis. At present no evidence has been obtained that would indicate the precise location within the context of these apparently silent regions, of genes encoding bacterial proteins shown to be contained by the transducing phage.

Note added in proof

The isolation of λ*aspC* transducing phages shown to encode r-protein S1 has recently been reported (Christiansen, L. & Pedersen, S. (1981) Mol. Gen. Genet. 181, 548-551). The identification of S1 coding capacity in this study rested initially upon the demonstrated migration of a species synthesised in λ*aspC*-infected cells to a position expected for S1 in the O'Farrell two dimensional polyacrylamide gel electrophoretic system. The identity was confirmed by a comparison of the patterns obtained in an SDS polyacrylamide gel electrophoretic analysis of partial chymotryptic digests of phage-encoded material and a pure S1 standard.

The close relationship between bacterial sequences contained by one such λ*aspC* phage, λ*aspC2*, and the λ*aroA* phage analysed in this study might be suggested by the similarity between their coding capacities. Among the bacterial products other than S1 demonstrated to be encoded by λ*aspC2*, two basic proteins of M_r = 80,000 and 26,000 would appear from their migration in O'Farrell two dimensional polyacrylamide gels to be identical to products encoded by λ*aroA* (Figure 3). A third acidic protein of M_r = 20,000 encoded by λ*aspC2* was not observed among λ*aroA*-encoded species, but may be a truncated or hybrid product.

Direct confirmation of the closeness of the relationship between chromosomal sequences contained by the two

phages is provided by a comparison of the restriction map of the λ*asp*C2 genome with that determined for phage λ*aro*A (Figure 6). Such a comparison clearly illustrates the identity of bacterial sequences contained by the two transducing phages, as defined by the distribution of Bam HI, Hin dIII, Sal I, Bgl II and Eco RI cleavage sites. The equivalence extends on the right from the att site, which indicates that the same site in the chromosome was used in the integration of the parent to λ*asp*C2 as in the integration of λ*aro*A, to a point 15 kbp leftward, where the insert in this λ*asp*C2 phage ends.

A detailed analysis of the chromosomal segment contained by λ*asp*C2, involving subcloning of restriction fragments of the insert DNA, has permitted localisation of the *rps*A gene to a 3 kbp Sal I/Bam HI fragment at the right junction of insert and phage sequences (Christiansen & Pedersen, 1981). This assignment strongly supports a location for the site of preferred λ integration in this chromosomal region immediately rightward of the *rps*A locus.

SUMMARY

The coding capacities of a λ*aroA* transducing phage have been analysed in an UVL irradiation and infection system by a combination of techniques of immunoprecipitation and SDS and O'Farrell gel electrophoresis. This analysis has indicated that six bacterial proteins are encoded by this phage, of M_r = 29,500-80,000. One of these species has been identified on the basis of immunological reactivity and migration in electrophoretic analyses as ribosomal protein S1, the product of the *rpsA* gene. Two other species have been identified as products of loci closely linked to *rpsA*, none appear to correspond to products of ribosomal protein genes as yet unmapped. The synthesis of five of these bacterial proteins in λ*aroA*-infected cells, including that identified as protein S1, has been shown to be directed by promoters of bacterial origin.

The physical structure of the genome of λ*aroA* has been analysed in considerable detail. This analysis has indicated that the bacterial insert carried by this phage is approximately 20 kbp in length. A comparison of the arrangement of bacterial sequences contained within this insert with that of the chromosomal context from which it was derived has suggested that the arrangement carried by the phage truly reflects that prevailing in the bacterial

chromosome. This observation indicates that the bacterial activity governing synthesis of S1 in the λ*aroA* context is likely to be the normal chromosomal promoter for the *rpsA* gene.

REFERENCES

(1) Dennis, P.P. (1974) J. Mol. Biol. 88, 25-41.

(2) Maaløe, O. & Kjeldgaard, N.O. (1966) in "Control of Macromolecular Synthesis" (Benjamin, W.A., NY).

(3) Dennis, P.P. & Nomura, M. (1974) Proc. Natl. Acad. Sci. USA 71, 3819-3823.

(4) Gausing, K. (1980) in "Ribosomes: Structure, Function and Genetics" Eds. Chambliss, G., Craven, G.R., Davies, J., Davis, K.M Kahan, L. & Nomura, M. (University Park Press, Baltimore, MD), pp. 693-718.

(5) Schrenk, W.J. & Weisberg, R.A. (1975) Mol. Gen. Genet. 137, 101-107.

(6) Shimada, K., Weisberg, R.A. & Gottesman, M.E. (1972) J. Mol. Biol. 63, 483-503.

(7) Kirschbaum, J.B. & Konrad, E.B. (1973) J. Bacteriol. 116, 517-526.

(8) Shizuya, H., Livingston, D.M. & Richardson, C.C. (1974) Proc. Natl. Acad. Sci. USA 71, 2614-2617.

(9) Jaskunas, S.R., Lindahl, L. & Nomura, M. (1975) Proc. Natl. Acad. Sci. USA 72, 6-10.

(10) Friesen, J.D., Parker, J., Watson, R.J., Fiil, N.P. & Pedersen, S. (1976) Mol. Gen. Genet. 144, 115-118.

(11) Kitakawa, M., Blumenthal, L. & Isono, K. (1980) Mol. Gen. Genet. 180, 343-349.

(12) Friesen, J.D., Parker, J., Watson, R.J., Bendiak, D., Reeh, S.V., Pedersen, S. & Fiil, N.P. (1976) Mol. Gen. Genet. 148, 93-98.

(13) Yamamoto, M., Strycharz, W.A. & Nomura, M. (1976) Cell 8, 129-138.

(14) Saito, H., Nakamura, Y. & Uchida, H. (1978) Mol. Gen. Genet. 165, 247-256.

(15) Gross, C.A., Blattner, F.R., Taylor, W.E., Lowe, P.A. & Burgess, R.R. (1979) Proc. Natl. Acad. Sci. USA 76, 5789-5793.

(16) Jaskunas, S.R., Fallon, A.M. & Nomura, M. (1977) J. Biol. Chem. 252, 7323-7336.

(17) Lindahl, L., Jaskunas, S.R., Dennis, P.P. & Nomura, M. (1975) Proc. Natl. Acad. Sci. USA 72, 2743-2747.

(18) Watson, R.J., Parker, J., Fiil, N.P., Flaks, J.G. & Friesen, J.D. (1975) Proc. Natl. Acad. Sci. USA 72, 2765-2769.

(19) Dabbs, E.R. (1980) J. Bacteriol. 144, 603-607.

(20) Isono, K. (1980) in "Ribosomes: Structure, Function & Genetics" pp. 641-669.

(21) Ono, M., Michihiko, K. & Mizushima, S. (1979) Mol. Gen. Genet. 174, 11-15.

(22) Nashimoto, H. & Uchida, H. (1975) J. Mol. Biol. 96, 443-453.

(23) Rosset, R. & Gorini, L. (1969) J. Mol. Biol. 39, 95-112.

(24) Apirion, D. & Schlessinger, D. (1968) J. Bacteriol. 96, 1431-1432.

(25) Isono, K. & Kitakawa, M. (1977) Mol. Gen. Genet. 153, 115-120.

(26) Leboy, P.E., Cox, E. & Flaks, J. (1964) Proc. Natl. Acad. Sci. USA 52, 1367-1372.

(27) Breckenridge, L. & Gorini, L. (1970) Genetics 6, 9-25.

(28) Kitakawa, M., Dabbs, E.R. & Isono, K. (1979) J. Bacteriol. 138, 832-838.

(29) Isono, K. (1978) Mol. Gen. Genet. 165, 265-268.

(30) Cannoh, M., Cabezon, T. & Bollen, A. (1974) Mol. Gen. Genet. 130, 321-326.

(31) deWilde, M., Michel, F. & Broman, K. (1974) Mol. Gen. Genet. 133, 329-333.

(32) Böck, A., Ruffler, D., Piepersberg, W. & Wittmann, H. G. (1974) Mol. Gen. Genet. 134, 325-332.

(33) Wittmann, H.G., Stöffler, G., Apirion, D., Rosen, L., Tanaka, K., Tamaki, M., Takata, R., Dekio, S., Otaka, E. & Osawa, S. (1973) Mol. Gen. Genet. 127, 175-189.

(34) Isono, K. & Kitakawa, M. (1978) Proc. Natl. Acad. Sci. USA 75, 6163-6167.

(35) Kitakawa, M. & Isono, K. (1977) Mol. Gen. Genet. 158, 149-155.

(36) Schnier, J. & Isono, K. (1979) Mol. Gen. Genet. 176, 313-318.

(37) Isono, K., Schnier, J. & Kitakawa, M. (1980) Mol. Gen. Genet. 179, 311-317.

(38) Dabbs, E.R. (1981) J. Bacteriol. 148, 379-382.

(39) Jaskunas, S.R., Fallon, A.M., Nomura, M., Williams, B.G. & Blattner, F.R. (1977) J. Biol. Chem. 252, 7355-7364.

(40) Jaskunas, S.R. & Nomura M. (1977) J. Biol. Chem. 252, 7337-7343.

(41) Linn, T. & Scaife, J. (1978) Nature 276, 33-37.

(42) Yamamoto, M. & Nomura, M. (1978) Proc. Natl. Acad. Sci. USA 75, 3891-3895.

(43) Yamamoto, M. & Nomura, M. (1979) J. Bacteriol. 137, 584-594.

(44) Newman, A.J., Linn, T.G. & Hayward, R.S. (1979) Mol. Gen. Genet. 169, 195-204.

(45) Lindahl, L., Zengel, J. & Nomura, M. (1977) J. Mol. Biol. 106, 837-856.

(46) Lindahl, L., Post, L., Zengel, J., Gilbert, S.F., Strycharz, W.A. & Nomura, M. (1977) J. Biol. Chem. 252, 7365-7383.

(47) Lindahl, L., Yamamoto, M., Nomura, M. Kirschbaum, J. B., Allet, B. & Rochaix, J.D. (1977) J. Mol. Biol. 109, 23-47.

(48) Post, L.E., Arfsten, A.E., Reusser, F. & Nomura, M. (1978) Cell 15, 215-219.

(49) Post, L.E. & Nomura, M. (1980) J. Biol. Chem. 255, 4660-4666.

(50) Post, L.E., Arfsten, A.E., Davis, G.R. & Nomura, M. (1980) J. Biol. Chem. 255, 4653-4659.

(51) Post, L.E. & Nomura, M. (1979) J. Biol. Chem. 254, 10604-10606.

(52) Post, L.E., Strycharz, G.D., Nomura, M., Lewis, H. & Dennis, P.P. (1979) Proc. Natl. Acad. Sci. USA 76, 1697-1701.

(53) Fallon, A.M., Jinks, C.S., Yamamoto, M. & Nomura, M. (1979) J. Bacteriol. 138, 383-396.

(54) Fallon, A.M., Jinks, C.S., Strycharz, G.D. & Nomura, M. (1979) Proc. Natl. Acad. Sci. USA 76, 3411-3415.

(55) Dennis, P.P. & Fiil, N.P. (1979) J. Biol. Chem. 254, 7540-7547.

(56) Dowsett, S.J. & Sells, B.H. (1980) Mol. Gen. Genet. 180, 331-341.

(57) Fukuda, R. (1980) Mol. Gen. Genet. 178, 483-486.

(58) Brot, N., Caldwell, P. & Weissbach, H. (1980) Proc. Natl. Acad. Sci. USA 77, 2592-2595.

(59) Wirth, R. & Böck, A. (1980) Mol. Gen. Genet. 178, 479-481.

(60) Yates, J.L., Arfsten, A.E. & Nomura, M. (1980) Proc. Natl. Acad. Sci. USA 77, 1837-1841.

(61) Yates, J.L. & Nomura, M. (1980) Cell 21, 517-522.

(62) Nomura, M. & Post, L.E. (1980) in "Ribosomes: Structure, Function and Genetics" pp. 671-691.

(63) Dennis, P.P. & Nomura, M. (1975) J. Mol. Biol. 97, 61-76.

(64) Dennis, P.P. (1977) J. Mol. Biol. 115, 603-625.

(65) Gausing, K. (1977) J. Mol. Biol. 115, 335-354.

(66) Maher, D.L. & Dennis, P.P. (1977) Mol. Gen. Genet. 155, 203-211.

(67) Chu, F., Kung, H-F., Caldwell, P., Weissbach, H. & Brot, N. (1976) Proc. Natl. Acad. Sci. USA 73, 3156-3159.

(68) Lindahl, L., Post, L.E. & Nomura M. (1976) Cell 9, 439-448.

(69) Wirth, R., Buckel, P. & Böck, A. (1977) FEBS Lett. 83, 103-106.

(70) Wittmann, H.G. (1974) in "Ribosomes. Eds. Nomura, M., Tissières, A. & Lengyel, P. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 93-114.

(71) Yokota, T., Arai, K. & Kaziro, Y. (1979) J. Biochem. (Japan) 86, 1725-1737.

(72) Tal, M., Aviram, M., Kanarek, A. & Weiss, A. (1971) Biochim. Biophys. Acta 222, 381-392.

(73) Szer, W., Hermoso, J.M. & Boublik, M. (1976) Biochem. Biophys. Res. Comm. 70, 957-964.

(74) Thomas, J.O., Kolb, A & Szer, W. (1978) J. Mol. Biol. 123, 163-176.

(75) Draper, D.E., Pratt, C.W. & von Hippel, P.H. (1977) Proc. Natl. Acad. Sci. USA 74, 4786-4790.

(76) Draper, D.E. & von Hippel, P.H. (1978) J. Mol. Biol. 122, 321-338.

(77) Draper, D.E. & von Hippel, P.H. (1978) J. Mol. Biol. 122, 339-359.

(78) Kamen, R. (1975) in "RNA phages" Ed. Zinder, N. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 203-234.

(79) Goelz, S. & Steitz, J.A. (1977) J. Biol. Chem. 252, 5177-5179.

(80) Miyajima, A. & Kaziro, Y. (1978) J. Biochem. (Japan) 83, 453-462.

(81) Adachi, K., Boyle, S.M. & Sells, B.H. (1980) J. Biol. Chem. 255, 357-360.

(82) Miller, J. (1972) in "Experiments in Molecular Genetics" (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 431.

(83) Ptashne, M. (1967) Proc. Natl Acad. Sci. USA 57, 306-313.

(84) Kaiser, A.D. (1957) Virology 3, 42-62.

(85) Appleyard, R.K. (1954) Genetics 39, 440-452.

(86) Mans, R.J. & Novelli, G.D. (1961) Arch. Biochem. Biophys. 94, 48-53.

(87) Laemmli, U.K. (1970) Nature 227, 680-685.

(88) Bonner, W.M. & Laskey, (1974) Eur. J. Biochem. 46, 83-88.

(89) O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007-4021.

(90) Kaiser, A.D. & Hogness, D.S. (1960) J. Mol. Biol. 2, 392-415.

(91) Marmur, J. (1961) J. Mol. Biol. 3, 208-218.

(92) Saito, H. & Miura, K-I. (1963) Biochim. Biophys. Acta 72, 619-629.

(93) Wahl, G.M., Stern, M. & Stark, G.R. (1979) Proc. Natl. Acad. Sci. USA 76, 3683-3687.

(94) Rigby, P.W.J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) J. Mol. Biol. 113, 237-251.

(95) Bloch, P.L., Phillips, T.A. & Neidhardt, F.C. (1980) J. Bacteriol. 141, 1409-1420.

(96) Szybalski, E.H. & Szybalski, W. (1979) Gene 7, 217-270.

(97) Daniels, D.L., DeWet, J.R. & Blattner, F.R. (1980) J. Virol. 33, 390-400.

(98) Nosikov, V.V., Braga, E.A. & Sain, B. (1978) Gene 4, 69-84.

(99) Li, K. & Subramanian, A.R. (1975) Anal. Biochem. 64, 121-129.

(100) Bachmann, B.J. & Low, K.B. (1980) Microbiol. Rev. 44, 1-56.

(101) Mutoh, N., Nagasawa, T. & Mizushima, S. (1981) J. Bacteriol. 145, 1085-1090.

(102) Yamamoto, M., Nomura, M., Ohsawa, H. & Maruo, B. (1977) J. Bacteriol. 132, 127-131.

(103) Anderson, J.J., Quay, S.C. & Oxender, D.L. (1976) J. Bacteriol. 126, 80-90.

(104) Clark, S.J., Low, B. & Konigsberg, W.H. (1973) J. Bacteriol. 113, 1091-1095.

(105) Gelfand, D.H. & Rudo, N. (1977) J. Bacteriol. 130, 441-444.

(106) Miller, H.I., Kikuchi, A., Nash, H.A., Weisberg, R. A. & Friedman, D.I. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 1121-1126.

(107) Ohsawa, H. & Maruo, B. (1976) J. Bacteriol. 127, 1157-1166.

(108) Reeve, E.C. & Doherty, P. (1968) J. Bacteriol. 96, 1450-1451.

(109) Sato, T & Yura, T. (1979) J. Bacteriol. 139, 468-477.

(110) Phillips, T.A., Bloch, P.L. & Neidhardt, F.C. (1980) J. Bacteriol. 144, 1024-1033.

(111) Nomura, M., Morgan, E.A. & Jaskunas, S.R. (1977) Annu. Rev. Genet. 11, 297-347.

(112) Laughrea, M. & Moore, P.B. (1978) J. Mol. Biol. 122, 109-112.

(113) Dzionara, M., Kaltschmidt, E. & Wittmann, H.G. (1970) Proc. Natl. Acad. Sci. USA 67, 1909-1913.

(114) Wittmann-Liebold, B. & Seib, C. (1979) FEBS Lett. 103, 61-65.

(115) Kaltschmidt, E & Wittmann, H.G. (1970) Anal. Biochem. 36, 401-412.

(116) Kessler, S.W. (1975) J. Immunol. 115, 1617-1624.

(117) Ivarie, R.D. & Jones, P.P. (1979) Anal. Biochem. 97, 24-35.

(118) Schaffner, W. & Weissmann, C. (1973) Anal. Biochem. 56, 502-514.

(119) Carmichael, G.G. (1975) J. Biol. Chem. 250, 6160-6169.

(120) Murialdo, H. (1971) in "Studies on Morphogenesis of Bacteriophage Lambda" Ph.D. thesis University of Toronto.

APPENDIX A : Immunoprecipitation of S1

Introduction

The main features of several sensitive immunoprecipitation methods (115,116) have been incorporated into a procedure designed to permit the detection of small quantities of r-protein S1 synthesised in UVL irradiated cells infected with transducing phages. Experiments described in the following section aim at establishing the conditions of antibody and immunoadsorbent concentration optimal to the use of this procedure.

Methods

Partial purification of antibody

Rabbit anti serum to E. coli r-protein S1 was cleared of aggregates by centrifugation at 10,000 rpm/15 min/4°C (Sorvall; SS34), then adjusted to 50% ammonium sulphate. The precipitated material was redissolved in phosphate buffered saline (PBS) containing; 25 mM potassium phosphate (pH 7.6), 0.1 M NaCl, and this solution was subjected to a second precipitation at 40% ammonium sulphate. The resultant precipitate, representing an enriched antibody fraction, was then redissolved in one-half the original volume of

PBS and dialysed twice for 3 hr at 4°C against 200 volumes of the same buffer supplemented with sodium azide (0.02%), before removing to storage at -70°C.

The protein concentration of this preparation was measured at 16 μg/ml using the method of Schaffner & Weissmann (117).

Treatment of immunoadsorbent

Protein A-sepharose CL-4B (5%) in NP40 buffer supplemented with sodium azide (0.02%), was stored at 4°C until required.

Preparation of radioactively labeled cell extract

A culture of strain S159 grown at 37°C in M9G medium (25 ml), was labeled over three generations with L-((U)-^{14}C) lysine at 2 μCi/ml (338 mCi/mmol). At A_{600} = 0.40 incorporation of label was halted by rapid chilling followed by the addition of a 100-fold excess of unlabeled lysine plus sodium azide (10 mM), and the cells collected by centrifugation at 10,000 rpm/10 min/4°C (Sorvall, SS34). The cell pellet was resuspended in 1.25 ml of 20 mM tris.HCl (pH 8.0) containing 25% sucrose, and a volume (250 μl) of a fresh 1:1 mixture of 25 mM $(Na)_2$EDTA (pH 8.0) plus lysozyme (2.5 mg/ml in 0.1 M tris.HCl, pH 8.0), added. After a 10 min incubation at 0°C, the suspension was treated with 0.5 ml of lysis solution (LS) for a further 15

min at 37°C. The resultant lysate was cleared of cell debris by centrifugation at 22,000 rpm/30 min/4°C (Beckman; 75Ti), then stored undiluted at -70°C until required.

Immunoprecipitation

Before reaction, both antibody preparation and radioactively labeled cell lysate were diluted as appropriate in PBS and NP40 buffer respectively, then cleared of aggregates by centrifugation at 22,000 rpm/30 min/4°C (Beckman 75Ti). Aliquots of the lysate (200 µl) adjusted to 5 mg/ml BSA (20 µl) and dispensed in Eppendorf tubes, were reacted with the antibody fraction (20 µl) in the desired ratio, during a 15 min incubation at 0°C. The reacted mixtures were then treated with appropriate volumes of protein A-sepharose freshly washed in NP40 buffer, and incubated for a further 15 min at 0°C. The immune complexes firmly associated with the adsorbent were pelleted at 12,800 g/60 sec and room temperature (Eppendorf microfuge), and washed six times in TSS buffer (1 ml) to remove contaminating radioactivity. The resulting precipitates were then solubilised in SDS-sample buffer by heating to 100°C for 4 min, and either the entire supernatant plus pellet transferred into 10 ml Aquasol for counting, or the supernatant free of adsorbent removed for storage at -70°C until required for SDS polyacrylamide gel electrophoretic analysis.

SDS polyacrylamide gel electrophoretic analysis of proteins

(See METHODS I).

Results

Immunotitration

Anti 31 serum and protein A-sepharose adsorbent were both titrated against a 1 in 50 dilution of a radioactively labeled crude cell lysate. The results of these titrations, presented graphically in Figure 10, clearly establish 3 μg antibody and 80-100 μl of adsorbent suspension (5%) as equivalence for this dilution of lysate. An increase in either antibody or adsorbent above these levels can be seen to result only in increased background contamination of precipitates.

SDS polyacrylamide gel electrophoretic analysis of immunoprecipitates

The nature of material precipitated from a 1 in 10 dilution of radioactively labeled cell lysate over a range of levels of antibody and adsorbent centred on equivalence was analysed by SDS polyacrylamide gel electrophoresis. The results of this analysis, shown in Figure 11, demonst-

Figure 10. Immunotitration of a radioactively labeled crude cell lysate with antibody and immunoadsorbent (inset).

In two separate experiments, volumes (200 µl) of a 1 in 50 dilution of a radioactively labeled cell lysate were titrated with antibody (0-10 µg) in a condition of adsorbent excess (120 µl of a 5% adsorbent suspension), and with immunoadsorbent (0-150 µl) in antibody excess (10 µg; Experiment 1, 6 µg; Experiment 2). The immunoprecipitates produced were washed then solubilised in SDS-sample buffer, and transferred completely into Aquasol for counting (all procedures as described in Methods).

The figures for antibody titration presented are corrected for cpm precipitated in the sample lacking antibody, while those for the titration of immunoadsorbent are corrected only for the buffer background.

The symbols ▽, ▼ indicate the equivalence points in each titration.

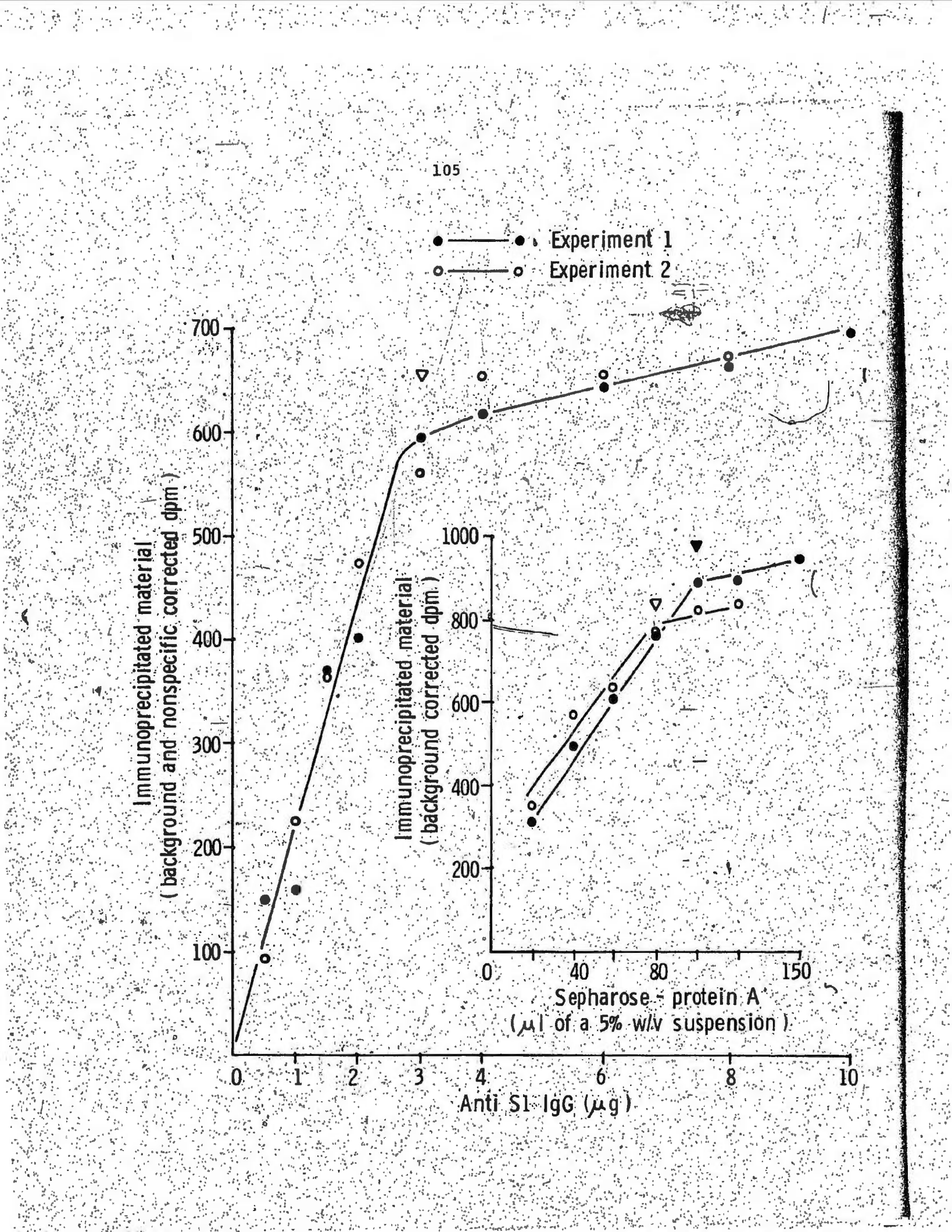
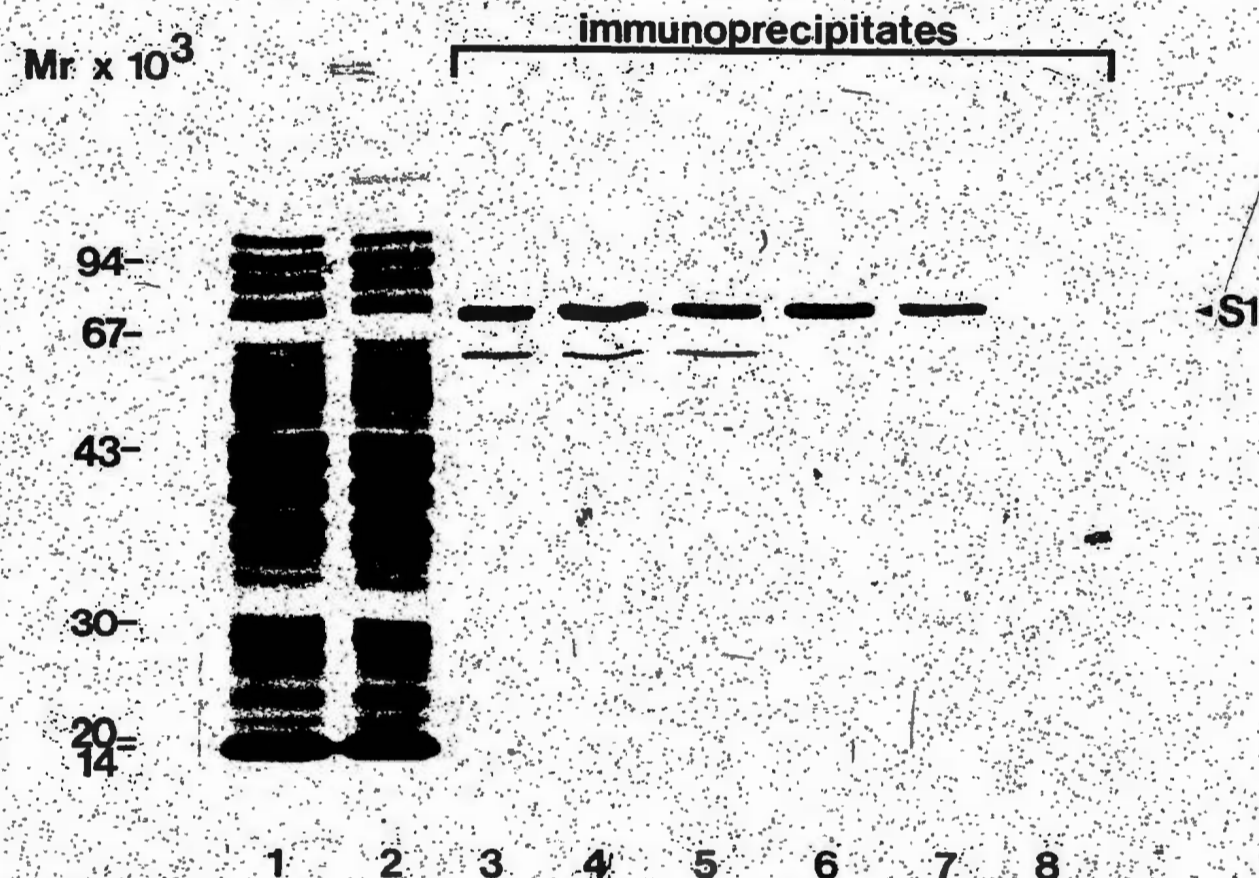

Experiment 1
Experiment 2
Immunoprecipitated material
(background and nonspecific corrected dpm)
700
600
500
400
300
200
100
0
1
2
3
4
6
8
10
Anti S1 IgG (μg)
Immunoprecipitated material
(background corrected dpm)
1000
800
600
400
200
0
40
80
150
Sepharose - protein A
(μl of a 5% w/v suspension)

Figure 11. SDS polyacrylamide gel electrophoresis of material immunoprecipitated from a radioactively labeled crude lysate of S159.

Volumes (200 µl) of a 1 in 10 dilution of radioactively labeled cell lysate were incubated with antibody (10-40 µg) and immunoadsorbent (150-500 µl of 5% adsorbent suspension) as in Figure 10. The immunoprecipitates produced were washed and treated with SDS-sample buffer to solubilise precipitated material. One-half volumes (50-100 µl) of these samples were removed for analysis in an SDS 10% acrylamide gel alongside total crude lysate (10 µl), and a sample (of equivalent activity) of material remaining after reaction of lysate with 20 µg antibody and 150 µl adsorbent suspension.

Following electrophoresis at 15 mA to stack and 30 mA to run (until the dye marker approached 1 cm from the lower end), the gel was stained and processed for autofluorography (all procedures as described in Methods). (Film exposure 48 hr). Molecular weights were determined using Pharmacia LMW protein markers. Lane 1 (35,700 cpm loaded) crude lysate; lane 2 (33,800 cpm) material remaining after reaction with anti S1 plus adsorbent; lane 3-8 immunoprecipitates, lane 3 (2,600 cpm) 20 µg anti S1, 500 µl adsorbent; lane 4 (2,700 cpm) 20 µg anti S1, 300 µl adsorbent; lane 5 (2,600 cpm) 20 µg anti S1, 150 µl adsorbent; lane 6 2,250 cpm) 10 µg anti S1, 150 µl adsorbent; lane 7 (1,650 cpm) 5 µg anti S1, 150 µl adsorbent; lane 8 (200 cpm) 150 µl adsorbent alone.


Mr x 10^3
immunoprecipitates
94–
67–
43–
30–
20–
14–
S1
1
2
3
4
5
6
7
8

rate by a comparison of the intensity of S1 and other bands evident in each immunoprecipitate sample analysed, that the addition of 10 µg antibody and 150 µl 5% adsorbent suspension to this lysate dilution serves to ensure quantitative precipitation of S1 with least contamination.

Discussion

The results of the previous section demonstrate that amounts of antibody and adsorbent suspension (5%) of 10 µg and 150 µl respectively provide for maximal sensitivity in the detection of low levels of S1 in a crude lysate of concentration equivalent to the 1 in 10 standard dilution. For this reason, these values of the two parameters were adopted in subsequent applications of the immunoprecipitation technique.

The nature of material other than protein S1 shown to be precipitated with this system includes a distinct group of proteins, perhaps representing poly(C)-binding species contaminating the original S1 challenge antigen (80 & 118). The choice of 10 µg antibody as the condition used in later uses of the immunoprecipitation system, reduces the problem posed to the detection of S1 synthesis by the precipitation of these species.

APPENDIX B : UVL irradiation of *E. coli* S159

Introduction

The UVL irradiation and infection system developed by Ptashne (82), in which irradiation of a UVL sensitive host at an appropriate level prior to infection is used to eliminate host chromosomal expression while leaving synthesis of phage proteins relatively unimpaired, has proved of considerable value in the identification of λ phage gene products. Experiments described in the following section aim at establishing the level of UVL exposure optimal for use of this system in identification of transducing phages containing the gene for ribosomal protein S1 of E. coli.

Methods

UVL irradiation of E. coli S159

Cultures of strain S159 grown at 37°C in M9M medium (30 ml), in dual label experiments with L-((U)-^{14}C) lysine (338 mCi/mmol) added to prelabel over three generations, were harvested at A_{600} = 0.40 by centrifugation at 10,000 rpm/10 min/4°C, and resuspended to A_{600} = 2.0 in M9M medium supplemented with 10 mM $MgSO_4$. A volume of cell

suspension (4 ml) transferred to a glass petri dish, was exposed evenly to UV light (3 J/m²/sec), and samples (100-200 µl) withdrawn to incubate at 37°C for 15 min with the simultaneous addition of TM buffer or λ helper (multiplicity of infection 5-10) (10-20 µl) as appropriate. At the end of this incubation period each culture was diluted 5-fold with prewarmed M9M (400-800 µl) containing either L-((U)-^{14}C) lysine (338 mCi/mmol) or in dual label experiments L-(4,5-^{3}H(N)) lysine (78.1 Ci/mmol), and incubated for a further 30 min at 37°C to pulse label. Labeling was then terminated by the addition of a 100-fold excess of unlabeled lysine plus sodium azide (10 mM) (55-110 µl), and samples taken either for measurement of total label incorporated into protein, or for SDS polyacrylamide gel electrophoretic analysis.

Determination of radioactivity incorporated into protein

(See METHODS I).

SDS polyacrylamide gel electrophoresis of proteins

(See METHODS I).

Immunoprecipitation of S1

(See METHODS I).

Results

UVL irradiation: Effect upon S1 synthesis

The effect of a range of UVL dosages upon the extent of residual host chromosome-directed S1 synthesis was determined. The results of this analysis, presented graphically in Figure 12, illustrate the severe impact of moderate to high levels of UVL irradiation upon synthesis both of S1 and of total protein. In cells irradiated at a dosage of 500 J/m^2, the extent of incorporation of radioactivity into total protein is only 1.5% that of unirradiated cells, while the amount of radioactivity precipitable with anti S1 serum is only 1.1% of the unirradiated control.

UVL irradiation: Effect upon phage protein synthesis

The effect of a range of UVL dosages upon the expression of phage proteins in irradiated cells was analysed. The analysis of total synthesis of protein, shown in Figure 13, demonstrates that phage infection of cells irradiated at moderate to high levels results in a marked net stimulation of protein synthesis over the uninfected control. Cells irradiated at 500 J/m^2, in which host chromosomal expression is only 1.5% of the unirradiated level, exhibit approximately 4-fold greater net incorporation of radioactivity in the infected compared to the uninfected condition. High-

Figure 12. Effect of UVL irradiation upon the synthesis of S1 and total protein in S159.

A culture of strain S159 grown in the presence of L-((U)-^{14}C) lysine at 0.1 μCi/ml was harvested, resuspended in M9M medium, and volumes (200 μl) withdrawn before irradiation, and after treatment with UVL at 100, 200, 500, 750 and 1,000 J/m^2. Following preincubation, cultures were diluted 5-fold with M9M (800 μl) containing L-(4,5-^{3}H(N)) lysine at 5 μCi/ml (zerodose-200 J/m^2) and 50 μCi/ml (500-1,000 J/m^2), and incubated at 37°C for 30 min. Samples (2 x 10 μl) were then removed for measurement of total incorporation of radioactivity into protein, and a volume (800 μl) of each culture harvested, extracted and extracts reacted with anti S1 serum to provide estimates of radioactivity incorporated into S1 (all procedures as described in Methods).

The values thus obtained for residual synthesis of total protein and S1 as presented, are in the form of (^{3}H)/(^{14}C) cpm ratios expressed as a percentage of the unirradiated ratio in each case.

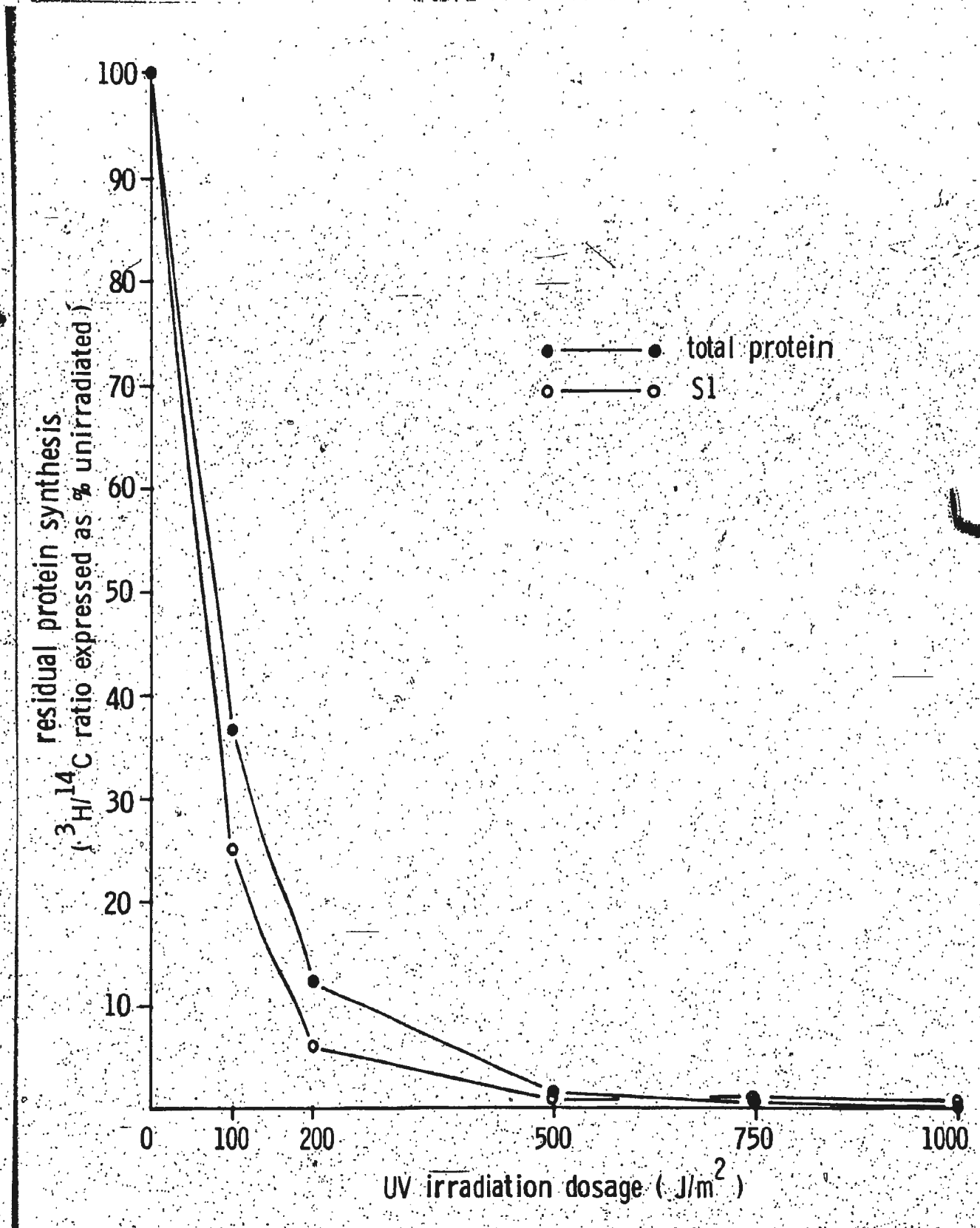
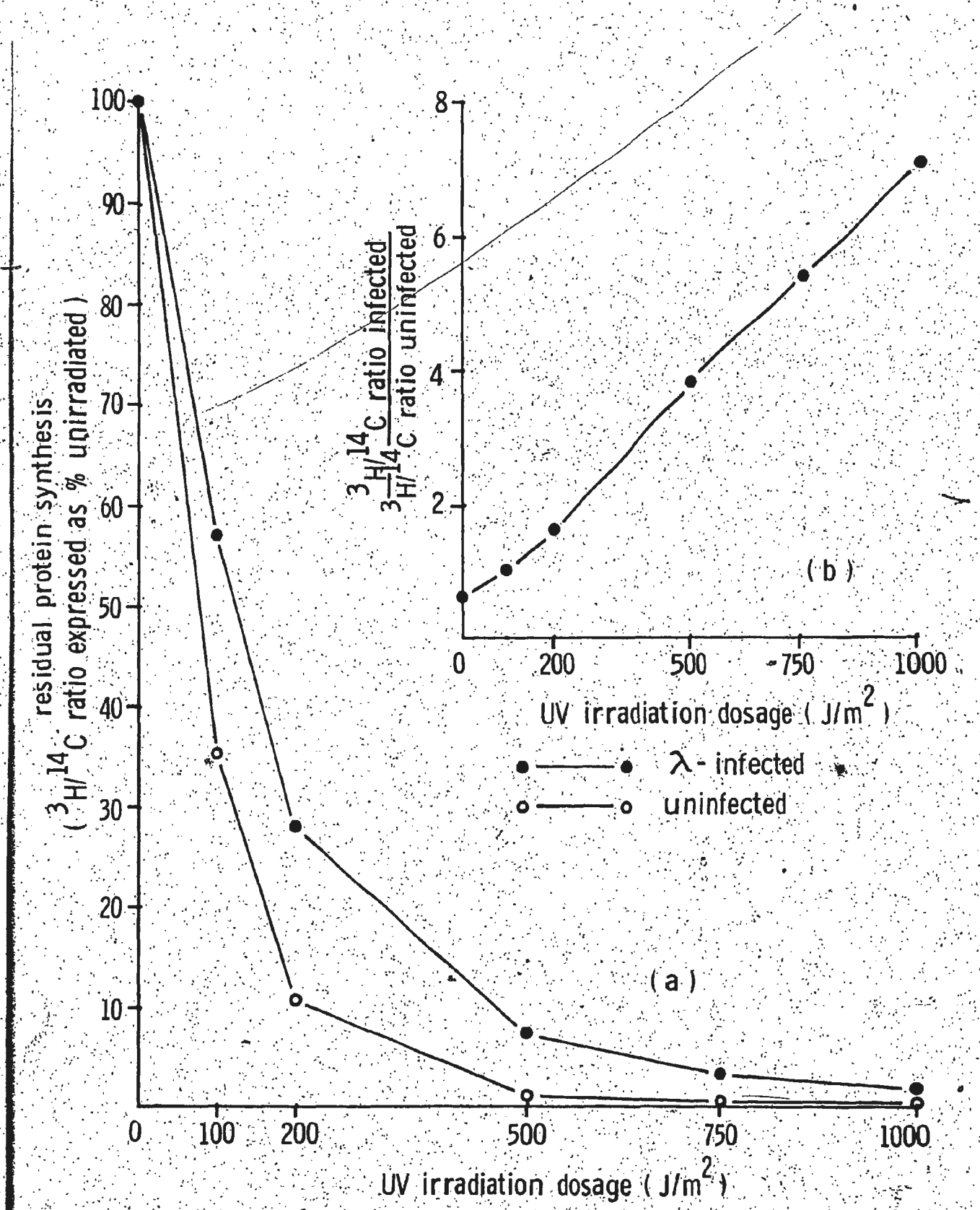

residual protein synthesis
($^3H/^{14}C$ ratio expressed as % unirradiated)
100
90
80
70
60
50
40
30
20
10
0
100
200
500
750
1000
UV irradiation dosage (J/m^2)
total protein
S1

Figure 13. Effect of UVL irradiation upon protein synthesis in λ helper-infected and uninfected S159.

A culture of strain S159 grown in the presence of L-((U)-^{14}C)-lysine at 0.05 μCi/ml, was harvested and resuspended in M9M medium containing 10 mM $MgSO_4$. Volumes (100 μl) of the culture were then removed before irradiation and after treatment with UVL at 100, 200, 500, 750 and 1,000 J/m^2, and preadsorbed with λ helper. Following preadsorption, each culture was diluted 5-fold in M9M medium (400 μl) containing L-(4,5-^{3}H(N)) lysine at 2.5 μCi/ml (zerodose-200 J/m^2) and 25 μCi/ml (500-1,000 J/m^2), and incubated at 37°C for 30 min. Samples were then withdrawn for determination of total incorporation of radioactivity into protein (all procedures as described in Methods).

The results of this analysis are presented both in the form of (^{3}H)/(^{14}C) cpm ratios for uninfected and infected samples expressed as a percentage of the unirradiated ratio in each case (a), and as a ratio relating the infected and uninfected (^{3}H)/(^{14}C) cpm ratios at each UVL dosage (b).


100
90
80
70
60
50
40
30
20
10
0
100
200
500
750
1000
$^3H/^{14}C$ residual protein synthesis ($^3H/^{14}C$ ratio expressed as % unirradiated)
UV irradiation dosage (J/m^2)
(a)
8
6
4
2
0
200
500
750
1000
$\frac{^3H/^{14}C \text{ ratio infected}}{^3H/^{14}C \text{ ratio uninfected}}$
UV irradiation dosage (J/m^2)
(b)
λ - infected
uninfected

er irradiating dosages enhance this stimulation (Figure 13 (b)), while reducing the absolute level of incorporation in both infected and uninfected cells (Figure 13 (a)).

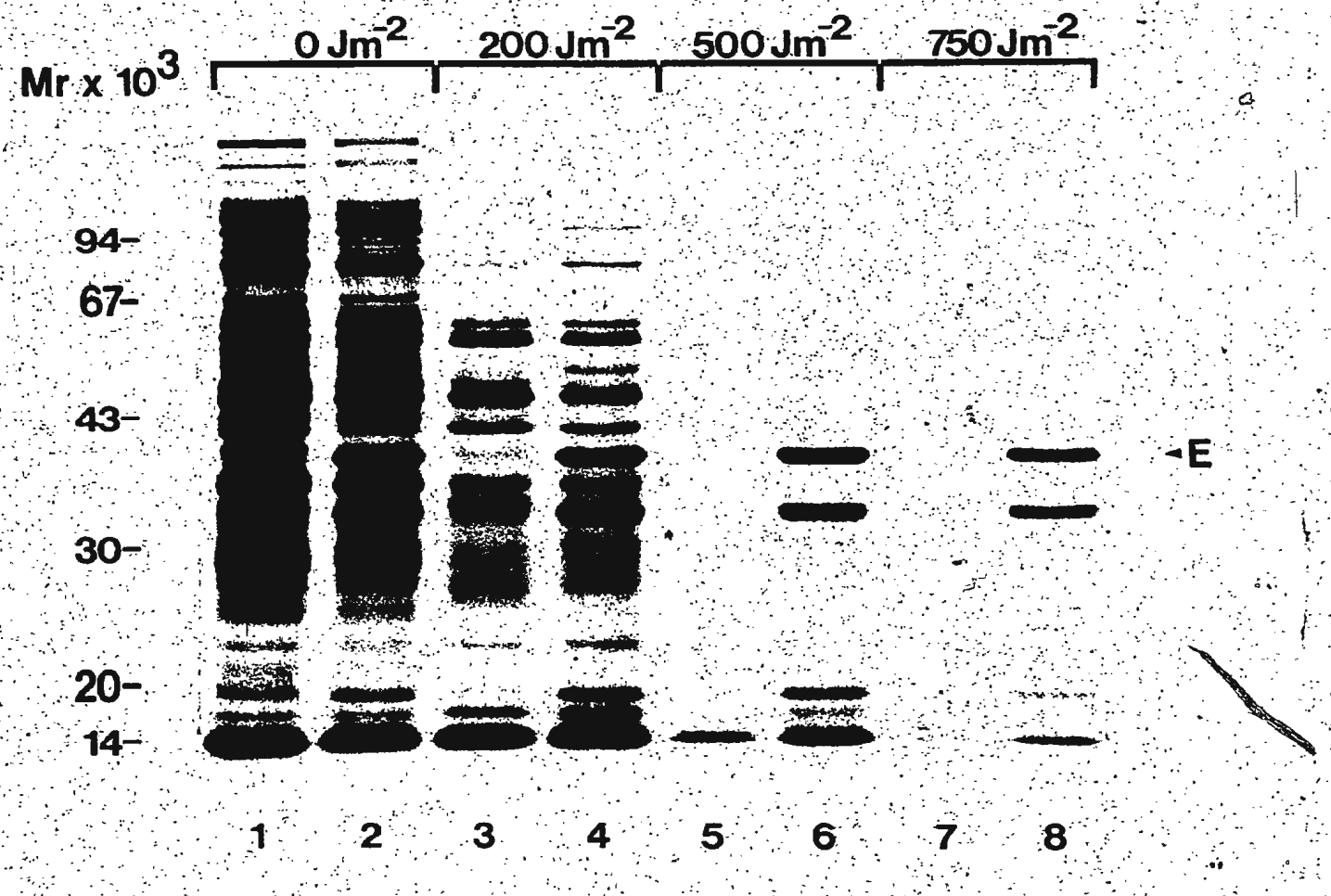
An SDS polyacrylamide gel electrophoretic analysis of proteins synthesised in infected and uninfected cells, shown in Figure 14, confirms the results of Figure 13 and in addition reveals the nature of proteins constituting residual host and phage-stimulated expression in cells irradiated over the range of UVL dosages. At 500 J/m^2, the only detectable synthesis in the uninfected condition is of a background of small molecular weight products, while in λ helper-infected cells synthesis of phage proteins is almost unimpaired. Higher irradiating dosages reduce the complexity of products synthesised in uninfected cells at the same time simplifying the identification of phage proteins synthesised in the infected condition.

Discussion

The results of the previous section demonstrate that cells irradiated with UVL at a level of 500 J/m^2 exhibit a severe reduction in host-directed synthesis of protein S1, while retaining relatively unimpaired the ability to suppo-

Figure 14. SDS polyacrylamide gel electrophoresis of proteins synthesised in uninfected and λ helper-infected S159.

A culture of strain S159 was grown, harvested and resuspended in M9M medium containing 10 mM $MgSO_4$, and sampled before and after UVL irradiation at 200, 500 and 750 J/m^2. Samples (150 µl) were preadsorbed with λ helper (15 µl) then diluted 5-fold in M9M (675 µl) containing L-((U)-^{14}C) lysine at 10 µCi/ml, and incubated at 37°C for 30 min. Volumes (500 µl) of each culture were precipitated with trichloroacetic acid, solubilised in SDS-sample buffer, and portions (5 µl, zerodose; 25 µl, 200-750 J/m^2) withdrawn for analysis. After electrophoresis at 15 mA to stack and 30 mA to run (until the dye marker approached 1 cm from the lower end), the gel was stained and processed for autofluorography (all procedures as described in Methods). (Film exposure 44 hr). Molecular weights were determined using Pharmacia LMW protein markers. Lane 1 (45,700 cpm loaded) unirradiated S159; lane 2 (38,100 cpm) unirradiated S159. λ helper; lane 3 (25,400 cpm) 200 J/m^2 S159; lane 4 (37,500 cpm) 200 J/m^2 S159. λ helper; lane 5 (5,560 cpm) 500 J/m^2 S159; lane 6 (16,300 cpm) 500 J/m^2 S159. λ helper; lane 7 (2,170 cpm) 750 J/m^2 S159; lane 8 (7,070 cpm) 750 J/m^2 S159. λ helper.


Mr x 10^3
0 Jm^-2
200 Jm^-2
500 Jm^-2
750 Jm^-2
94-
67-
43-
30-
20-
14-
E
1
2
3
4
5
6
7
8

en UVL dosage and amount of host chromosome and phage-stimulated expression is essentially identical to that determined previously for the UVL-sensitive strain C159 (119). In light of these observations, a level of UVL exposure of 500 J/m^2 was adopted for use in future experiments designed to permit detection of transducing phage-encoded synthesis of S1 in the UVL irradiation and infection system.